



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/68, C12N 5/02, 5/06, 15/00, 15/64, C07H 21/04		A1	(11) International Publication Number: WO 98/14614
			(43) International Publication Date: 9 April 1998 (09.04.98)
(21) International Application Number: PCT/US97/17791 (22) International Filing Date: 3 October 1997 (03.10.97) (30) Priority Data: 08/726,867 4 October 1996 (04.10.96) US 08/728,963 11 October 1996 (11.10.96) US 08/907,598 8 August 1997 (08.08.97) US (71) Applicant: LEXICON GENETICS INCORPORATED [US/US]; 4000 Research Forest Drive, The Woodlands, TX 77381 (US). (72) Inventors: SANDS, Arthur ; 163 Bristol Bend Circle, The Woodlands, TX 77382 (US). FRIEDRICH, Glenn ; 30 Reflection Point, The Woodlands, TX 77381 (US). ZAMBROWICZ, Brian ; 18 Firethorne Place, The Woodlands, TX 77382 (US). BRADLEY, Allan ; 5127 Queensloch, Houston, TX 77096 (US). (74) Agents: CORUZZI, Laura, A. et al. ; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).		(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, HU, ID, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: AN INDEXED LIBRARY OF CELLS CONTAINING GENOMIC MODIFICATIONS AND METHODS OF MAKING AND UTILIZING THE SAME			
<div style="text-align: center;"> <p>VICTR 1: SA Puromycin PA</p> <p>VICTR 2: SA Puromycin SD</p> <p>VICTR 3: PGK promoter Puromycin SD</p> <p>VICTR 4: PGK promoter Puromycin SD SA STOPS SD</p> <p>VICTR 5: PGK promoter Puromycin SD SA STOPS polyA</p> </div>			
(57) Abstract <p>Methods and vectors (both DNA and retroviral) are provided for the construction of a Library of mutated cells. The Library will preferably contain mutations in essentially all genes present in the genome of the cells. The nature of the Library and the vectors allow for methods of screening for mutations in specific genes, and for gathering nucleotide sequence data from each mutated gene to provide a database of tagged gene sequences. Such a database provides a means to access the individual mutant cell clones contained in the Library. The invention includes the described Library, methods of making the same, and vectors used to construct the Library. Methods are also provided for accessing individual parts of the Library either by sequence or by pooling and screening. The invention also provides for the generation of non-human transgenic animals which are mutant for specific genes as isolated and generated from the cells of the Library.</p>			

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**AN INDEXED LIBRARY OF CELLS CONTAINING GENOMIC MODIFICATIONS
AND METHODS OF MAKING AND UTILIZING THE SAME**

The present application claims priority to U.S.
5 Applications Ser. Nos. 08/726,867, filed October 4, 1996,
08/728,963, filed October 11, 1996, and 08/907,598, filed
August 8, 1997, the disclosures of which are herein
incorporated by reference.

10 **1.0. FIELD OF THE INVENTION**

The invention relates to an indexed library of
genetically altered cells and methods of organizing the cells
into an easily manipulated and characterized Library. The
invention also relates to methods of making the library,
15 vectors for making insertion mutations in genes, methods of
gathering sequence information from each member clone of the
Library, and methods of isolating a particular clone of
interest from the Library.

20 **2.0. BACKGROUND OF THE INVENTION**

The general technologies of targeting mutations into the
genome of cells, and the process of generating mouse lines
from genetically altered embryonic stem (ES) cells with
specific genetic lesions are well known (Bradley, 1991, Cur.
25 Opin. Biotech. 2:823-829). A random method of generating
genetic lesions in cells (called gene, or promoter, trapping)
has been developed in parallel with the targeted methods of
genetic mutation (Allen et al., 1988 Nature 333(6176):852-
855; Brenner et al., 1989, Proc. Natl. Acad. Sci. U.S.A.
30 86(14):5517-5521; Chang et al., 1993, Virology 193(2):737-
747; Friedrich and Soriano, 1993, Insertional mutagenesis by
retroviruses and promoter traps in embryonic stem cells, p.
681-701. In Methods Enzymol., vol. 225., P. M. Wassarman and
M. L. DePamphilis (ed.), Academic Press, Inc., San Diego;
35 Friedrich and Soriano, 1991, Genes Dev. 5(9):1513-1523;
Gossler et al., 1989, Science 244(4903):463-465; Kerr et al.,
1989, Cold Spring Harb. Symp. Quant. Biol. 2:767-776; Reddy
et al., 1991, J Virol. 65(3):1507-1515; Reddy et al., 1992,

Proc. Natl. Acad. Sci. U.S.A. 89(15):6721-6725; Skarnes et al., 1992, Genes Dev. 6(6):903-918; von Melchner and Ruley, 1989, J. Virol. 63(8):3227-3233; Yoshida et al., 1995, Transgen. Res. 4:277-287). Gene trapping provides a means to
5 create a collection of random mutations by inserting fragments of DNA into transcribed genes. Insertions into transcribed genes are selected over the background of total insertions since the mutagenic DNA encodes an antibiotic resistance gene or some other selectable marker. The
10 selectable marker lacks its own promoter and enhancer and must be expressed by the endogenous sequences that flank the marker after it has integrated. Using this approach, transcription of the selectable marker is activated and the cell gene is concurrently mutated. This type of strict
15 selection makes it possible to easily isolate thousands of ES cell colonies, each with a unique mutagenic insertion.

Collecting mutants on a large-scale has been a powerful genetic technique commonly used for organisms which are more amenable to such analysis than mammals. These organisms,
20 such as *Drosophila melanogaster*, yeast *Saccharomyces cerevisiae*, and plants such as *Arabidopsis thaliana* are small, have short generation times and small genomes (Bellen et al., 1989, Genes Dev. 3(9):1288-1300; Bier et al., 1989, Genes Dev. 3(9):1273-1287; Hope, 1991, Develop. 113(2):399-408.
25 These features allow an investigator to rear many thousands or millions of different mutant strains without requiring unmanageable resources. However, these type of organisms have only limited value in the study of biology relevant to human physiology and health. It is therefore important to
30 have the power of large-scale genetic analysis available for the study of a mammalian species that can aid in the study of human disease. Given that the entire human genome is presently being sequenced, the comprehensive genetic analysis of a related mammalian species will provide a means to
35 determine the function of genes cloned from the human genome. At present, rodents, and particularly mice, provide the best model for genetic manipulation and analysis of mammalian

physiology.

Gene trapping has been used as an analytical tool to identify genes and regulatory regions in a variety of animal cell types. One system that has proved particularly useful is based on the use of ROSA (reverse orientation splice acceptor) retroviral vectors (Friedrich and Soriano, 1991 and 1993).

The ROSA system can generate mutations that result in a detectable homozygous phenotype with a high frequency. About 50% of all the insertions caused embryonic lethality. The specifically mutated genes may easily be cloned since the gene trapping event produces a fusion transcript. This fusion transcript has trapped exon sequences appended to the sequences of the selectable marker allowing the latter to be used as a tag in polymerase chain reaction (PCR)-based protocols, or by simple cDNA cloning. Examples of genes isolated by these methods include a transcription factor related to human TEF-1 (transcription enhancer factor-1) which is required in the development of the heart (Chen et al., 1994, Genes Devel. 8:2293-2301. Another (spock), is distantly related to yeast genes encoding secretion proteins and is important during gastrulation.

The above experiments have established that the ROSA system is an effective analytical tool for genetic analysis in mammals. However, the structure of many ROSA vectors selects for the "trapping" of 5' exons which, in many cases, do not encode proteins. Such a result is adequate where one wishes to identify and eventually clone control (i.e., promoter or enhancer) sequences, but is not optimal where the generation of insertion-inactivated null mutations is desired, and relevant coding sequence is needed. Thus, the construction of large-scale mutant (preferably null mutant) libraries requires the use of vectors that have been designed to select for insertion events that have occurred within the coding region of the mutated genes as well as vectors that are not limited to detecting insertions into expressed genes.

3.0. SUMMARY OF THE INVENTION

An object of the present invention is to provide a set of genetically altered cells (the 'Library'). The genetic alterations are of sufficient randomness and frequency such
5 that the combined population of cells in the Library represent mutations in essentially every gene found in the cell's genome. The Library is used as a source for obtaining specifically mutated cells, cell lines derived from the individually mutated cells, and cells for use in the
10 production of transgenic non-human animals.

A further object is to provide the vectors, both DNA and retroviral based, that may be used to generate the Library. Typically, at least two distinct vector designs will be used in order to mutate genes that are actively expressed in the
15 target cell, and genes that are not expressed in the target cell. Combining the mutant cells obtained using both types of vectors best ensures that the Library provides a comprehensive set of gene mutations.

A particularly useful vector class contemplated by the
20 present invention includes a vector for inserting foreign exons into animal cell transcripts that comprises a selectable marker, a promoter element operatively positioned 5' to the selectable marker, a splice donor site operatively positioned 3' to the selectable marker, and a second
25 mutagenic foreign polynucleotide sequence located upstream from the promoter element that disrupts, or otherwise "poisons", the splicing or read-through expression of the endogenous cellular transcript. Typically, the mutagenic foreign polynucleotide sequence may incorporate a
30 polyadenylation (pA) site, a nested set of stop codons in each of the three reading frames, splice acceptor and splice donor sequences in operable combination, a mutagenic exon, or any mixture of mutagenic features that effectively prevent the expression of the cellular gene. For example, a
35 polyadenylation sequence may be incorporated in addition to or in lieu of the splice donor sequence. A preferred organization for the mutagenic polynucleotide sequence

comprises a polyadenylation site positioned upstream from a selectable marker which is in turn located upstream from a splice acceptor sequence. Preferably, such a vector does not comprise a transcription terminator or polyadenylation site operatively positioned relative to the coding region of the selectable marker, and shall not comprise a splice acceptor site operatively positioned between the promoter element and the initiation codon of said selectable marker.

An additional vector contemplated by the present invention is designed to replace the normal 3' end of an animal cell transcript with a foreign exon. Such a vector shall generally be engineered to comprise a selectable marker, a splice acceptor site operatively positioned upstream (5') from the initiation codon of the selectable marker, and a polyadenylation site operatively positioned downstream (3') from the termination codon (3' end) of the selectable marker. Preferably, the vector will not comprise a promoter element operatively positioned upstream from the coding region of the selectable marker, and will not comprise a splice donor sequence operatively positioned between the 3' end of the coding region of the selectable marker and the polyadenylation site.

Yet another vector contemplated by the present invention is a vector designed to insert a mutagenic foreign polynucleotide sequence within an animal cell transcript (i.e., the foreign polynucleotide sequence is flanked on both sides by endogenous exons). As described above, the mutagenic foreign polynucleotide sequence may be any sequence that disrupts the normal expression of the gene into which the vector has integrated. Optionally, the vector may additionally incorporate a selectable marker, a splice acceptor site operatively positioned 5' to the initiation codon of the selectable marker, a splice donor site operatively positioned 3' to said selectable marker. Preferably, this vector shall not comprise a polyadenylation site operatively positioned 3' to the coding region of said selectable marker, and shall not comprise a promoter element

operatively positioned 5' to the coding region of said selectable marker.

An additional embodiment of the present invention is a library of genetically altered cells that have been treated 5 to stably incorporate one or more types of the vectors described above. The presently described library of cultured animal cells may be made by a process comprising the steps of treating (*i.e.*, infecting, transfecting, retrotransposing, or virtually any other method of 10 introducing polynucleotides into a cell) a population of cells to stably integrate a vector that mediates the splicing of a foreign exon internal to a cellular transcript, transfecting another population of cells to stably integrate a vector that mediates the splicing of a foreign exon 5' to 15 an exon of a cellular transcript, and selecting for transduced cells that express the products encoded by the foreign exons.

Alternatively, an additional embodiment of the present invention describes a mammalian cell library made by a method 20 comprising the steps of: transfecting a population of cells with a vector capable of expressing a selectable marker in the cell only after the vector inserts into the host genome; transfecting or infecting a population of cells with a vector containing a selectable marker that is substantially only 25 expressed by cellular control sequences (after the vector integrates into the host cells genome); and growing the transfected cells under conditions that select for the expression of the selectable marker.

In an additional embodiment of the present invention, 30 the two populations of transfected cells will be individually grown under selective conditions, and the resulting mutated population of cells collectively comprises a substantially comprehensive library of mutated cells.

In an additional embodiment of the present invention, 35 the individual mutant cells in the library are separated and clonally expanded. Additionally, the clonally expanded mutant cells may then be analyzed to ascertain the DNA

sequence, or partial DNA sequence of the mutated host gene.

The presently described methods of making, organizing, and indexing libraries of mutated animal cells are also broadly applicable to virtually any eukaryotic cells that may
5 be genetically manipulated and grown in culture.

The invention provides for sequencing every gene mutated in the Library. The resulting sequence database subsequently serves as an index for the library. In essence, every cell line in the Library is individually catalogued using the
10 partial sequence information. The resulting sequence is specific for the mutated gene since the present methods are designed to obtain sequence information from exons that have been spliced to the marker sequence. Since the coverage of the mutagenesis is preferably the entire set of genes in the
15 genome, the resulting Library sequence database contains sequence from essentially every gene in the cell. From this database, a gene of interest can be identified. Once identified, the corresponding mutant cell may be withdrawn from the Library based on cross reference to the sequence
20 data.

An additional embodiment of the invention provides for methods of isolating mutations of interest from the Library. Two methods are proposed for obtaining individual mutant cell lines from the Library. The first provides a scheme where
25 clones of the cells generated using the above vectors are pooled into sets of defined size. Using the procedure described below which utilizes reverse transcription (RT) and polymerase chain reaction (PCR), a cell line with a mutation in a gene whose sequence is partly or wholly known is
30 isolated from organized sets of these pools. A few rounds of this screening procedure results in the isolation of the desired individual cell line.

A second procedure involves the sequencing of regions flanking the vector insertion sites in the various cells in
35 the library. The sequence database generated from these data effectively constitutes an index of the clones in the library that may be used to identify cells having mutations in

specific genes.

4.0. DESCRIPTION OF THE FIGURES

Figure 1. Shows a diagrammatic representation of 5 different vectors that are generally representative of the type of vectors that may be used in the present invention.

Figure 2. Shows a general strategy for identifying "trapped" cellular sequences by PCR analysis of the cellular exons that flank the foreign intron introduced by the VICTR 2 vector.

Figure 3 shows a PCR based strategy for identifying tagged genes by chromosomal location.

Figure 4. Is a diagrammatic representation of a strategy of identifying or indexing the specific clones in the library via PCR analysis and sequencing of mRNA samples obtained from the cells in the library.

Figure 5. Is a diagrammatic representation of a method of isolating positive clones by screening pooled mutant cell clones.

Figure 6. Partial nucleic acid or predicted amino acid sequence data from 9 clones (OST1-9) isolated using the described techniques aligned with similar sequences from previously characterized genes.

Figure 7. Provides a diagrammatic representation of VICTRs 3 and 20 as well as the transcripts that result after integration into a hypothetical region of the target cell genome (i.e., "Wildtype Locus").

Figure 8. Provides a representative list of a portion of the known genes that have been identified using the disclosed methods and technology.

5.0. DETAILED DESCRIPTION OF THE INVENTION

The present invention describes a novel indexed library containing a substantially comprehensive set of mutations in the host cell genome, and methods of making and using the same. The presently described Library comprises as a set of cell clones that each possess at least one mutation (and preferably a single mutation) caused by the insertion of DNA that is foreign to the cell. For the purposes of the present invention, "foreign" polynucleotide sequences can be any sequences that are newly introduced to a cell, do not naturally occur in the cell at the engineered region of the chromosome, or occur in the cell but are not organized to provide an identical function to that provided in the engineered vector.

The particularly novel features of the Library include the methods of construction, and indexing. To index the library, the mutant cells of the library are clonally expanded and each mutated gene is at least partially sequenced. The Library thus provides a novel tool for assessing the specific function of a given gene. The insertions cause a mutation which allow for essentially every gene represented in the Library to be studied using genetic techniques either *in vitro* or *in vivo* (via the generation of transgenic animals). For the purposes of the present invention, the term "essentially every gene" shall refer to the statistical situation where there is generally at least about a 70 percent probability that the genomes of cells used to construct the library collectively contain at least one inserted vector sequence in each gene, preferably a 85 percent probability, and more specifically at least about a 95 percent probability as determined by a standard Poisson distribution.

Also for the purposes of the present invention the term "gene" shall refer to any and all discrete coding regions of the cell's genome, as well as associated noncoding and regulatory regions. Additionally, the term operatively positioned shall refer to the control elements or genes that

are provided with the proper orientation and spacing to provide the desired or indicated functions of the control elements or genes.

For the purposes of the present invention, a gene is "expressed" when a control element in the cell mediates the production of functional or detectable levels of mRNA encoded by the gene, or a selectable marker inserted therein. A gene is not expressed where the control element in the cell is absent, has been inactivated, or does not mediate the production of functional or detectable levels of mRNA encoded by the gene, or a selectable marker inserted therein.

5.1. Vectors used to build the Library

A number of investigators have developed gene trapping vectors and procedures for use in mouse and other cells (Allen et al., 1988; Bellen et al., 1989, Genes Dev. 3(9):1288-1300; Bier et al., 1989, Genes Dev. 3(9):1273-1287; Bonnerot et al., 1992, J Virol. 66(8):4982-4991; Brenner et al., 1989; Chang et al., 1993; Friedrich and Soriano, 1993; Friedrich and Soriano, 1991; Goff, 1987, Methods Enzymol. 152:469-481; Gossler et al.; Hope, 1991; Kerr et al., 1989; Reddy et al., 1991; Reddy et al., 1992; Skarnes et al., 1992; von Melchner and Ruley; Yoshida et al., 1995). The gene trapping system described in the present invention is based on significant improvements to the published SA (splice acceptor) DNA vectors and the ROSA (reverse orientation, splice acceptor) retroviral vectors (Chen et al., 1994; Friedrich and Soriano, 1991 and 1993). The presently described vectors also use a selectable marker called β geo. This gene encodes a protein which is a fusion between the β -galactosidase and neomycin phosphotransferase proteins. The presently described vectors place a splice acceptor sequence upstream from the β geo gene and a poly-adenylation signal sequence downstream from the marker. The marker is integrated after transfection by, for example, electroporation (DNA vectors), or retroviral infection, and gene trap events are selected based on resistance to G418

resulting from activation of β geo expression by splicing from the endogenous gene into the ROSA splice acceptor. - This type of integration disrupts the transcription unit and preferably results in a null mutation at the locus.

- 5 Although gene trapping has proven a useful analytical tool, the present invention contemplates gene trapping on a large scale. The vectors utilized in the present invention have been engineered to overcome the shortcomings of the early gene trap vector designs, and to facilitate procedures
10 allowing high throughput. In addition, procedures are described that allow the rapid and facile acquisition of sequence information from each trapped cDNA which may be adapted to allow complete automation. These latter procedures are also designed for flexibility so that
15 additional molecular information can easily be obtained subsequently. The present invention therefore incorporates gene trapping into a larger and unique tool. A specially organized set of gene trap clones that provide a novel and powerful new tool of genetic analysis.
- 20 The presently described vectors are superficially similar to the ROSA family of vectors, but constitute significant improvements and provide for additional features that are useful in the construction and indexing of the Library. Typically, gene trapping vectors are designed to
25 detect insertions into transcribed gene regions within the genome. They generally consist of a selectable marker whose normal expression is handicapped by exclusion of some element required for proper transcription. When the vector integrates into the genome, and acquires the necessary
30 element by juxtaposition, expression of the selectable marker is activated. When such activation occurs, the cell can survive when grown in the appropriate selective medium which allows for the subsequent isolation and characterization of the trapped gene. Integration of the gene trap generally
35 causes the gene at the site of integration to be mutated.

Some gene trapping vectors have a splice acceptor preceding a selectable marker and a poly-adenylation signal

following the selectable marker, and the selectable marker gene has its own initiator ATG codon. Using this arrangement, the fusion transcripts produced after integration generally only comprise exons 5' to the insertion site to the known marker sequences. Where the vector has inserted into the 5' region of the gene, it is often the case that the only exon 5' to the vector is a non-coding exon. Accordingly, the sequences obtained from such fusions do not provide the desired sequence information about the relevant gene products. This is because untranslated sequences are generally less well conserved than coding sequences.

To compensate for the short-comings of earlier vectors, the vectors of the present invention have been designed so that 3' exons are appended to the fusion transcript by replacing the poly-adenylation and transcription termination signals of earlier ROSA vectors with a splice donor (SD) sequence. Consequently transcription and splicing generally results in a fusion between all or most of the endogenous transcript and the selectable marker exon, for example β geo, neomycin (neo) or puromycin (puro). The exon sequences immediately 3' to the selectable marker exon may then be sequenced and used to establish a database of expressed sequence tags. The presently described procedures will typically provide approximately 200 nucleotides of sequence, or more. These sequences will generally be coding and therefore informative. The prediction that the sequence obtained will be from coding region is based on two factors. First, gene trap vectors are generally found near the 5' end of the gene immediately after untranslated exons because the method selects for integration events that place the initiator ATG of the selectable marker as the first encountered, and thus used, for translation. Second, mammalian transcripts have short 5' untranslated regions (UTRs) which are typically between 50 and 150 nucleotides in length.

The obtained sequence information also provides a ready source of probes that may be used to isolate the full-length

gene or cDNA from the host cell, or as heterologous probes for the isolation of homologous genes in other species.

Internal exons in mammalian transcripts are generally quite small, on the average 137 bases with few over 300 5 bases. Consequently, a large internal exon may be spliced less efficiently. Thus, the presently described vectors have been designed to sandwich relatively small selectable markers (for example: neo, ~800 bases, or a smaller drug resistance gene such as puro, ~600 bases) between the requisite splicing 10 elements to produce relatively small exons. Exons of this size are more typical of mammalian exons and do not present undue problems for the splicing machinery of the cell. Such a design consideration is novel to the presently disclosed gene trapping vectors. Accordingly, an additional embodiment 15 of the claimed vectors is that the respective splice acceptor and splice donor sites are engineered such that they are operatively positioned close to the ends of the selectable marker coding region (the region spanning from the initiation codon to the termination codon). Generally, the splice 20 acceptor or splice donor sequences shall appear within about 80 bases from the nearest end of the selectable marker coding region, preferably within about 50 bases from the nearest end of the coding region, more preferably within about 30 bases from the nearest end of the coding regions and specifically 25 within about 20 bases of the nearest end of the selectable marker coding region.

The new vectors are represented in retroviral form in Figure 1. They are used by infecting target cells with retroviral particles such that the proviruses shown in the 30 schematic can be found in the genome of the target. These vectors are called VICTR which is an acronym for "viral constructs for trapping".

The presently described retroviral vectors may be used in conjunction with retroviral packaging cell lines such as 35 those described in U.S. Patent No. 5,449,614 ("'614 patent") issued September 12, 1995, herein incorporated by reference. Where non-mouse animal cells are to be used as targets for

generating the described libraries, packaging cells producing retrovirus with amphotropic envelopes will generally be employed to allow infection of the host cells.

The mutagenic gene trap DNA may also be introduced into the target cell genome by various transfection techniques which are familiar to those skilled in the art such as electroporation, lipofection, calcium phosphate precipitation, infection, retrotransposition, and the like. Examples of such techniques may be found in Sambrook et al. (1989) Molecular Cloning Vols. I-III, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, and Current Protocols in Molecular Biology (1989) John Wiley & Sons, all Vols. and periodic updates thereof, herein incorporated by reference. The transfected versions of the retroviral vectors are typically plasmid DNA molecules containing DNA cassettes comprising the described features between the retroviral LTRs.

The vectors VICTR 1 and 2 (Fig. 1) are designed to trap genes that are transcribed in the target cell. To trap genes that are not expressed in the target cell, gene trap vectors such as VICTR 3, 4 and 5 (described below) are provided. These vectors have been engineered to contain a promoter element capable of initiating transcription in virtually any cell type which is used to transcribe the coding sequence of the selectable marker. However, in order to get proper translation of the marker product, and thus render the cell resistant to the selective antibiotic, a polyadenylation signal and a transcription termination sequence must be provided. Vectors VICTR 3 through 5 are constructed such that an effective polyadenylation signal can only be provided by splicing with an externally provided downstream exon that contains a poly-adenylation site. Therefore, since the selectable marker coding region ends only in a splice donor sequence, these vectors must be integrated into a gene in order to be properly expressed. In essence, these vectors append the foreign exon encoding the marker to the 5' end of an endogenous transcript. These events will tag genes and

create mutations that are used to make clones that will become part of the Library.

With the above design considerations, the VICTR series of vectors, or similarly designed and constructed vectors, have the following features. VICTR 1 is a terminal exon gene trap. VICTR 1 does not contain a control region that effectively mediates the expression of the selectable marker gene. Instead, the coding region of the selectable marker contained in VICTR 1, in this case encoding puromycin resistance (but which can be any selectable marker functional in the target cell type), is preceded by a splice acceptor sequence and followed by a polyadenylation addition signal sequence. The coding region of the puro gene has an initiator ATG which is downstream and adjacent to a region of sequence that is most favorable for translation initiation in eukaryotic cells - the so called Kozak consensus sequence (Kozak, 1989, J. Cell, Biol. 108(2):229-241). With a Kozak sequence and an initiator ATG, the puro gene in VICTR 1 is activated by integrating into the intron of an active gene, and the resulting fusion transcript is translated beginning at the puromycin initiation (ATG/AUG) codon. However, terminal gene trap vectors need not incorporate an initiator ATG codon. In such cases, the gene trap event requires splicing and the translation of a fusion protein that is functional for the selectable marker activity. The inserted puromycin coding sequence must therefore be translated in the same frame as the "trapped" gene.

The splice acceptor sequence used in VICTR 1 and other members of the VICTR series is derived from the adenovirus major late transcript splice site located at the intron 1/exon 2 boundary. This sequence contains a polypyrimidine stretch preceding the AG dinucleotide which denotes the actual splice site. The presently described vectors contemplate the use of any similarly derived splice acceptor sequence. Preferably, the splice acceptor site will only rarely, if ever, be involved in alternative splicing events.

The polyadenylation signal at the end of the *puro* gene is derived from the bovine growth hormone gene. Any similarly derived polyadenylation signal sequence could be used if it contains the canonical AATAAA and can be demonstrated to terminate transcription and cause a polyadenylate tail to be added to the engineered coding exons.

VICTR 2 is a modification of VICTR 1 in which the polyadenylation signal sequence is removed and replaced by a splice donor sequence. Like VICTR 1, VICTR 2 does not contain a control region that effectively mediates the expression of the selectable marker gene. Typically, the splice donor sequence to be employed in a VICTR series vector shall be determined by reference to established literature or by experimentation to identify which sequences properly initiate splicing at the 5' end of introns in the desired target cell. The specifically exemplified sequence, AGGTAAGT, results in splicing occurring in between the two G bases. Genes trapped by VICTR 2 splice upstream exons onto the *puro* exon and downstream exons onto the end of the *puro* exon. Accordingly, VICTR 2 effectively mutates gene expression by inserting a foreign exon in-between two naturally occurring exons in a given transcript. Again, the *puro* gene may or may not contain a consensus Kozak translation initiation sequence and properly positioned ATG initiation codon. As discussed above, gene trapping by VICTR 1 and VICTR 2 requires that the mutated gene is expressed in the target cell line. By incorporating a splice donor into the VICTR traps, transcript sequences downstream from the gene trap insertion can be determined. As described above, these sequences are generally more informative about the gene mutated since they are more likely to be coding sequences. This sequence information is gathered according to the procedures described below.

VICTR 3, VICTR 4 and VICTR 5 are gene trap vectors that do not require the cellular expression of the endogenous trapped gene. The VICTR vectors 3 through 5 all comprise a

promoter element that ensures that transcription of the selectable marker would be found in all cells that have taken up the gene trap DNA. This transcription initiates from a promoter, in this case the promoter element from the mouse 5 phosphoglycerate kinase (PGK) gene. However, since the constructs lack a polyadenylation signal there can be no proper processing of the transcript and therefore no translation. The only means to translate the selectable marker and get a resistant cell clone is by acquiring a 10 polyadenylation signal. Since polyadenylation is known to be concomitant with splicing, a splice donor is provided at the end of the selectable marker. Therefore, the only positive gene trap events using VICTR 3 through 5 will be those that integrate into a gene's intron such that the marker exon is 15 spliced to downstream exons that are properly polyadenylated. Thus genes mutated with the VICTR vectors 3 through 5 need not be expressed in the target cell, and these gene trap vectors can mutate all genes having at least one intron. The design of VICTR vectors 3 through 5 requires a promoter 20 element that will be active in the target cell type; a selectable marker and a splice donor sequence. Although a specific promoter was used in the specific embodiments, it should be understood that appropriate promoters may be selected that are known to be active in a given cell type. 25 Typically, the considerations for selecting the splice donor sequence are identical to those discussed for VICTR 2, *supra*. VICTR 4 differs from VICTR 3 only by the addition of a small exon upstream from the promoter element of VICTR 4. This exon is intended to stop normal splicing of the mutated 30 gene. It is possible that insertion of VICTR 3 into an intron might not be mutagenic if the gene can still splice between exons, bypassing the gene trap insertion. The exon in VICTR 4 is constructed from the adenovirus splice acceptor described above and the synthetic splice donor also described 35 above. Stop codons are placed in all three reading frames in the exon, which is about 100 bases long. The stops would truncate the endogenous protein and presumably cause a

mutation.

A conceptually similar alternative design uses a terminal exon like that engineered into VICTR 5. Instead of a splice donor, a polyadenylation site is used to terminate transcription and produce a truncated message. Stops in all three frames are also provided to truncate the endogenous protein as well as the resulting transcript.

VICTR 20 is a modified version of VICTR 3 that incorporates a polyadenylation site 5' to the PGK promoter, the IRES β geo sequence (i.e., foreign mutagenic polynucleotide sequence) 5' to the polyadenylation site, and a splice acceptor site 5' to the IRES β geo coding region. VICTR 20 additionally incorporates, in operable combination, a pair of recombinase recognition sites that flank the PGKpuroSD cassette.

All of the traps of the VICTR series are designed such that a fusion transcript is formed with the trapped gene. For all but VICTR 1, the fusion contains cellular exons that are located 3' to the gene trap insertion. All of the flanking exons may be sequenced according to the methods described in the following section. To facilitate sequencing, specific sequences are engineered onto the ends of the selectable marker (e.g., puromycin coding region). Examples of such sequences include, but are not limited to unique sequences for priming PCR, and sequences complementary to the standard M13 forward sequencing primer. Additionally, stop codons are added in all three reading frames to ensure that no anomalous fusion proteins are produced. All of the unique 3' primer sequences are followed immediately by the synthetic 9 base pair splice donor sequence. This keeps the size of the exon comprising the selectable marker (puro gene) at a minimum to best ensure proper splicing, and positions the amplification and sequencing primers immediately adjacent to the flanking "trapped" exons to be sequenced as part of the construction of a Library database.

When any members of the VICTR series are constructed as retroviruses, the direction of transcription of the

selectable marker is opposite to that of the direction of the normal transcription of the retrovirus. The reason for this organization is that the transcription elements such as the polyadenylation signal, the splice sites and the promoter

5 elements found in the various members of the VICTR series interfere with the proper transcription of the retroviral genome in the packaging cell line. This would eliminate or significantly reduce retroviral titers. The LTRs used in the construction of the packaging cell line are self-

10 inactivating. That is, the enhancer element is removed from the 3' U3 sequences such that the proviruses resulting from infection would not have an enhancer in either LTR. An enhancer in the provirus may otherwise affect transcription of the mutated gene or nearby genes.

15 Since a 'cryptic' splice donor sequence is found in the inverted LTRs, this splice donor sequence has been removed from the VICTR vectors by site specific mutagenesis. It was deemed necessary to remove this splice donor so that it would not affect the trapping splicing events.

20 The present disclosure also describes vectors that incorporate a new way to conduct positive selection. VICTR 3 and VICTR 20 are two examples of such vectors. Both VICTR 3 and VICTR 20, contain PGKpuroSD which must splice into exons of gene that provide a polyadenylation addition sequence in

25 order to allow expression of the puromycin selectable marker gene. When placed in a targeting vector, PGKpuroSD allows for positive selection when targeting takes place. In addition to providing positive selection, targeted events among resistant colonies are easy to identify by the 3' RACE

30 protocols (see section 5.2.2., *infra*) used for Omnibank production. This automated process allows for the rapid identification of targeted events. It is important that unlike SA β geo, PGKpuroSD does not require expression of the targeted gene in order to provide positive selection. In

35 addition, VICTR 20 provides 2 potential positive selectable markers (puro and neo). The use of two selectable markers, when a gene is expressed, provides a means to increase the

targeting efficiency by requiring both selectable markers to function which is much more remote a possibility than having one selectable marker function unless there is a targeted event. The addition of a negative selection cassette to 5 these vectors would only increase their targeting efficiency.

An additional feature that may be incorporated into the presently described vectors includes the use of recombinase recognition sequences. Bacteriophage P1 Cre recombinase and flp recombinase from yeast plasmids are two examples of 10 site-specific DNA recombinase enzymes which cleave DNA at specific target sites (loxP sites for cre recombinase and frt sites for flp recombinase) and catalyze a ligation of this DNA to a second cleaved site. When a piece of DNA is flanked by 2 loxP or frt sites (e.g., recombinase control elements) 15 in the same orientation, the corresponding recombinase will cause the removal of the intervening DNA sequence. When a piece of DNA is flanked by loxP or frt sites in an indirect orientation, the corresponding recombinase will essentially activate the control elements to cause the intervening DNA to 20 be flipped into the opposite orientation. These recombinases provide powerful approaches for manipulating DNA *in situ*.

Recombinases have important applications for gene trapping and the production of a library of trapped genes. When constructs containing PGKpuroSD are used to trap genes, 25 the fusion transcript between puromycin and sequences of the trapped gene could result in some level of protein expression from the trapped gene if translational reinitiation occurs. Another important issue is that several reports suggest that the PGK promoter can affect the expression of nearby genes. 30 These effects may make it difficult to determine gene function after a gene trap event since one could not discern whether a given phenotype is associated with the inactivation of a gene, or the transcription of nearby genes. Both potential problems are solved by exploiting recombinase 35 activity. When PGKpuroSD is flanked by loxP, frt, or any other recombinase sites in the same orientation, the addition of the corresponding recombinase will result in the removal

of PGKpuroSD. In this way, effects caused by PGKpuroSD fusion transcripts, or the PGK promoter, are avoided.

Accordingly, a vector that may be particularly useful for the practice of the present invention is VICTR 20. This vector replaces the terminal exon of VICTR 5 with a splice acceptor located upstream from the β geo gene which can be used for both LacZ staining and antibiotic selection. The fusion gene possesses its own initiator methionine and an internal ribosomal entry site (IRES) for efficient translation initiation. In addition, the PGK promoter and puromycin-splice donor sequences have been flanked by lox P recombination sites. This allows for the ability to both remove and introduce sequences at the integration site and is of potential value with regard to the manipulation of regions proximal to trapped target genes (Barinaga, Science 265:26-8, 1994). While this particular vector includes lox P recombination sites, the present invention is in no way limited to the use of this specific recombination site (Akagi et al., Nucleic Acids Res 25:1766-73, 1997).

Another very important use of recombinases is to produce mutations that can be made tissue-specific and/or inducible. In the presently described vectors, the SA β geo or SAIRES β geo component provides the mutagenic function by "trapping" the normal splicing from preceding exons. If the SA β geo is flanked by inverted loxP, frt, or any other recombinase sites, the addition of the corresponding recombinase results in the flipping of the SA β geo sequence so that it no longer prevents the normal splicing of the cellular gene into which it is integrated. To make a gene trap tissue-specific or inducible one could produce the trap with SA β geo in the reverse orientation and then provide recombinase activity only at the time and place where one wishes to remove the gene function. The use of tissue-specific or inducible recombinase constructs allows one to choose when and where one removes, or activates, the function of the targeted gene.

One method for practicing the inducible forms of recombinase mediated gene expression involves the use of

vectors that use inducible or tissue specific promoter/operator elements to express the desired recombinase activity. The inducible expression elements are preferably operatively positioned to allow the inducible control or
5 activation of expression of the desired recombinase activity. Examples of such inducible promoters or control elements include, but are not limited to, tetracycline, metallothionine, ecdysone, and other steroid-responsive promoters, rapamycin responsive promoters, and the like (No
10 et al., Proc Natl Acad Sci USA 93:3345-51, 1996; Furth et al., Proc Natl Acad Sci USA 91:9302-6, 1994). Additional control elements that can be used include promoters requiring specific transcription factors such as viral, particularly HIV, promoters. Vectors incorporating such promoters would
15 only express recombinase activity in cells that express the necessary transcription factors.

The incorporation of recombinase sites into the gene trapping vectors highlights the value of using the described gene trap vectors to deliver specific DNA sequence elements
20 throughout the genome. Although a variety of vectors are available for placing sequences into the genome, the presently described vectors facilitate both the insertion of the specific elements, and the subsequent identification of where sequence has inserted into the cellular chromosome.
25 Additionally, the presently described vectors may be used to place recombinase recognition sites throughout the genome. The recombinase recognition sites could then be used to either remove or insert specific DNA sequences at predetermined locations.

30 Moreover, the described gene trap vectors can also be used to insert regulatory elements throughout the genome. Recent work has identified a number of inducible or repressible systems that function in the mouse. These include the rapamycin, tetracycline, ecdysone,
35 glucocorticoid, and heavy metal inducible systems. These systems typically rely on placing DNA elements in or near a promoter. An inducible or repressible transcription factor

that can identify and bind to the DNA element may also be engineered into the cells. The transcription factor will specifically bind to the DNA element in either the presence or absence of a ligand that binds to the transcription factor and, depending on the structure of the transcription factor, it will either induce or repress the expression of the cellular gene into which the DNA elements have been inserted. The ability to place these inducible or repressible elements throughout the genome would increase the value of the library by adding the potential to regulate the expression of the trapped gene.

The vectors described also have important applications for the overexpression of genes or portions of genes to select for phenotypic effects. Currently, overexpression of cDNA libraries to look for genes or parts of genes with specific functions is a common practice. One example would be to overexpress genes or portions of genes to look for expression that causes loss of contact inhibition for cell growth as determined by growth in soft agar. This would allow the identification of genes or portions of genes that can act as oncogenes. Simple modifications of VICTR 20 would allow it to be used for these applications. For example, the addition of an internal ribosome entry site (IRES) 3' to the puromycin selectable marker and before the SD sequence, would result in the overexpression of sequences from the trapped downstream exons. In addition, the IRES could be modified by, for example, the addition of one or two nucleotides such that there could be 3 basic vectors that would allow expression of trapped exons in all three reading frames. In this way, genes could be trapped throughout the genome resulting in overexpression of genes, or portions thereof, to examine the cellular function of the trapped genes. This identification of function could be done by selecting for the function of interest (i.e., growth in soft agar could result from the overexpression of potentially oncogenic genes). This technique would allow for the screening or selection of large numbers of genes, or portions thereof, by

overexpressing the genes and identifying cells displaying the phenotypes of interest. Additional assays could, for example, identify candidate tumor suppressor genes based on their ability, when overexpressed, to prevent growth in soft agar.

Given the fact that expression pattern information can provide insight into the possible functions of genes mutated by the current methods, another LTR vector, VICTR 6, has been constructed in a manner similar to VICTR 5 except that the terminal exon has been replaced with either a gene coding for β -galactosidase (β gal) or a fusion between β -gal and neomycin phosphotransferase (β geo), each preceded by a splice acceptor and followed by a polyadenylation signal. Endogenous gene expression and splicing of these markers into cellular transcripts and translation into fusion proteins will allow for increased mutagenicity as well as the delineation of expression through Lac Z staining.

An additional vector, VICTR 12, incorporates two separate selectable markers for the analysis of both integration sites and trapped genes. One selectable marker (e.g. puro) is similar to that for VICTRs 3 through 5 in that it contains a promoter element at its 5' end and a splice donor sequence 3'. This gene cassette is located in the LTRs of the retroviral vector. The other marker (neo) also contains a promoter element but has a polyadenylation signal present at the 3' end of the coding sequence and is positioned between the viral LTRs. Both selectable markers contain an initiator ATG for proper translation. The design of VICTR 12 allows for the assessment of absolute titer as assayed by the number of colonies resistant to antibiotic selection for the constitutively expressed marker possessing a polyadenylation signal. This titer can then be compared to that observed for gene-trapping and stable expression of the resistance marker flanked at its 3' end by a splice donor. These numbers are important for the calculation of gene trapping frequency in the context of both nonspecific binding by retroviral integrase and directed binding by chimeric

integrase fusions. In addition, it provides an option to focus on the actual integration sites through infection and selection for the marker containing the polyadenylation signal. This eliminates the need for the fusion protein binding to occur upstream and in the proximity of the target gene. Theoretically, any transcription factor binding sites present within the genome are targets for proximal integration and subsequent antibiotic resistance. Analysis of sequences flanking the LTRs of the retroviral vector should reveal canonical factor binding sites. In addition, by including the promoter/splice donor design of VICTR 3, gene-trapping abilities are retained in VICTR 12.

VICTR A is a vector which does not contain gene trapping constructs but rather a selectable marker possessing all of the required entities for constitutive expression including, but not limited to, a promoter element capable of driving expression in eukaryotic cells and a polyadenylation and transcriptional terminal signal. Similar to VICTR 12, downstream gene trapping is not necessary for successful selection using VICTR A. This vector is intended solely to select for successful integrations and serves as a control for the identification of transcription factor binding sites flanking the integrant as mentioned above.

Finally, VICTR B is similar to VICTR A in that it comprises a constitutively expressed selectable marker, but it also contains the bacterial β -lactamase ampicillin resistance selectable marker and a ColE1 origin of replication. These entities allow for the rapid cloning of sequences flanking the long terminal repeats through restriction digestion of genomic DNA from infected cells and ligation to form plasmid molecules which can be rescued by bacterial transformation, and subsequently sequenced. This vector allows for the rapid analysis of cellular sequences that contain putative binding sites for the transcription factor of interest.

Other vector designs contemplated by the present invention are engineered to include an inducible regulatory

elements such as tetracycline, ecdysone, and other steroid-responsive promoters (No et al., Proc Natl Acad Sci USA 93:3345-51, 1996; Furth et al., Proc Natl Acad Sci USA 91:9302-6, 1994). These elements are operatively positioned 5 to allow the inducible control of expression of either the selectable marker or endogenous genes proximal to site of integration. Such inducibility provides a unique tool for the regulation of target gene expression.

All of the gene trap vectors of the VICTR series, with 10 the exception of VICTRs A and B, are designed to form a fusion transcript between vector encoded sequence and the trapped target gene. All of the flanking exons may be sequenced according to the methods described in the following section. To facilitate sequencing, specific sequences are 15 engineered onto the ends of the selectable marker (e.g., puromycin coding region). Examples of such sequences include, but are not limited to unique sequences for priming PCR, and sequences complementary to standard M13 sequencing primers. Additionally, stop codons are added in all three 20 reading frames to ensure that no anomalous fusion proteins are produced. All of the unique 3' primer sequences are immediately followed by a synthetic 9 base pair splice donor sequence. This keeps the size of the exon comprising the selectable marker at a minimum to ensure proper splicing, and 25 positions the amplification and sequencing primers immediately adjacent to the flanking trapped exons to be sequenced as part of the generation of the collection of cells representing mutated transcription factor targets.

Since a cryptic splice donor sequence is found in the 30 inverted LTRs, this cryptic splice donor sequence has been removed from the VICTR vectors by site specific mutagenesis. It was deemed necessary to remove this splice donor so that it would not affect trapping associated splicing events.

When any members of the VICTR series are packaged into 35 infectious virus, the direction of transcription of the selectable marker is opposite to that of the direction of the normal transcription of the retrovirus. The reason for this

organization is that the regulatory elements such as the polyadenylation signal, the splice sites and the promoter elements found in the various members of the VICTR series can interfere with the transcription of the retroviral genome in the packaging cell line. This potential interference may significantly reduce retroviral titers.

Although specific gene trapping vectors have been discussed at length above, the invention is by no means to be limited to such vectors. Several other types of vectors that may also be used to incorporate relatively small engineered exons into a target cell transcripts include, but are not limited to, adenoviral vectors, adenoassociated virus vectors, SV40 based vectors, and papilloma virus vectors. Additionally, DNA vectors may be directly transferred into the target cells using any of a variety of biochemical or physical means such as lipofection, chemical transfection, retrotransposition, electroporation, and the like.

Although, the use of specific selectable markers has been disclosed and discussed herein, the present invention is in no way limited to the specifically disclosed markers. Additional markers (and associated antibiotics) that are suitable for either positive or negative selection of eukaryotic cells are disclosed, *inter alia*, in Sambrook et al. (1989) Molecular Cloning Vols. I-III, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, and Current Protocols in Molecular Biology (1989) John Wiley & Sons, all Vols. and periodic updates thereof, as well as Table I of U.S. Patent No. 5,464,764 issued November 7, 1995, the entirety of which is herein incorporated by reference. Any of the disclosed markers, as well as others known in the art, may be used to practice the present invention.

5.2. The Analysis of Mutated Genes and Transcripts

The presently described invention allows for large-scale genetic analysis of the genomes of any organism for which there exists cultured cell lines. The Library may be constructed from any type of cell that can be transfected by

standard techniques or infected with recombinant retroviral vectors.

Where mouse ES cells are used, then the Library becomes a genetic tool able to completely represent mutations in essentially every gene of the mouse genome. Since ES cells can be injected back into a blastocyst and become incorporated into normal development and ultimately the germ line, the cells of the Library effectively represent a complete panel of mutant transgenic mouse strains (see generally, U.S. Patent No. 5,464,764 issued November 7, 1995, herein incorporated by reference).

A similar methodology may be used to construct virtually any non-human transgenic animal (or animal capable of being rendered transgenic). Such nonhuman transgenic animals may include, for example, transgenic pigs, transgenic rats, transgenic rabbits, transgenic cattle, transgenic goats, and other transgenic animal species, particularly mammalian species, known in the art. Additionally, bovine, ovine, and porcine species, other members of the rodent family, e.g. rat, as well as rabbit and guinea pig and non-human primates, such as chimpanzee, may be used to practice the present invention.

Transgenic animals produced using the presently described library and/or vectors are useful for the study of basic biological processes and diseases including, but not limited to, aging, cancer, autoimmune disease, immune disorders, alopecia, glandular disorders, inflammatory disorders, diabetes, arthritis, high blood pressure, atherosclerosis, cardiovascular disease, pulmonary disease, degenerative diseases of the neural or skeletal systems, Alzheimer's disease, Parkinson's disease, asthma, developmental disorders or abnormalities, infertility, epithelial ulcerations, and microbial pathogenesis (a relatively comprehensive review of such pathogens is provided, *inter alia*, in Mandell et al., 1990, "Principles and Practice of Infectious Disease" 3rd. ed., Churchill Livingstone Inc., New York, N.Y. 10036, herein incorporated

by reference). As such, the described animals and cells are particularly useful for the practice of functional genomics.

5.2.1. Constructing a Library of Individually Mutated Cell Clones

The vectors described in the previous section were used to infect (or transfect) cells in culture, for example, mouse embryonic stem (ES) cells. Gene trap insertions were initially identified by antibiotic resistance (e.g., puromycin). Individual clones (colonies) were moved from a culture dish to individual wells of a multi-welled tissue culture plate (e.g. one with 96 wells). From this platform, the clones were be duplicated for storage and subsequent analysis. Each multi-well plate of clones was then processed by molecular biological techniques described in the following section in order to derive sequence of the gene that has been mutated. This entire process is presented schematically in Figure 4 (described below).

5.2.2. Identifying and Sequencing the Tagged Genes in the Library.

The relevant nucleic acid (and derived amino acid sequence information) will largely be obtained using PCR-based techniques that rely on knowing part of the sequence of the fusion transcripts (see generally, Frohman et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85(23):8998-9000, and U.S. Patents Nos. 4,683,195 to Saiki et al., and 4,683,202 to Mullis, which are herein incorporated by reference). Typically, such sequences are encoded by the foreign exon containing the selectable marker. The procedure is represented schematically in Figure 2 (3' RACE). Although each step of the procedure may be done manually, the procedure is also designed to be carried out using robots that can deliver reagents to multi well culture plates (e.g., but not limited to, 96-well plates).

The first step generates single stranded complementary DNA which is used in the PCR amplification reaction (Figure

2). The RNA substrate for cDNA synthesis may either be total cellular RNA or an mRNA fraction; preferably the latter. mRNA was isolated from cells directly in the wells of the tissue culture dish. The cells were lysed and mRNA was bound
5 by the complementary binding of the poly-adenylate tail to a poly-thymidine-associated solid matrix. The bound mRNA was washed several times and the reagents for the reverse transcription (RT) reaction were added. cDNA synthesis in the RT reaction was initiated at random positions along the
10 message by the binding of a random sequence primer (RS). This RS primer has approximately 6-9 random nucleotides at the 3' end to bind sites in the mRNA to prime cDNA synthesis, and a 5' tail sequence of known composition to act as an anchor for PCR amplification in the next step. There is
15 therefore no specificity for the trapped message in the RT step. Alternatively, a poly-dT primer appended with the specific sequences for the PCR may be used. Synthesis of the first strand of the cDNA initiates at the end of each trapped gene. At this point in the procedure, the bound mRNA may be
20 stored (at between about -70° C and about 4° C) and reused multiple times. Such storage is a valuable feature where one subsequently desires to analyze individual clones in more detail. The bound mRNA may also be used to clone the entire transcript using PCR-based protocols.

25 Specificity for the trapped, fusion transcript is introduced in the next step, PCR amplification. The primers for this reaction are complementary to the anchor sequence of the RS primer and to the selectable marker. Double stranded fragments between a fixed point in the selectable marker gene
30 and various points downstream in the appended transcript sequence are amplified. It is these fragments which will become the substrates for the sequencing reaction. The various end-points along the transcript sequence were determined by the binding of the random primer during the RT
35 reaction. These PCR products were diluted into the sequencing reaction mix, denatured and sequenced using a primer specific for the splice donor sequences of the gene

trap exon. Although, standard radioactively labeled nucleotides may be used in the sequencing reactions, sequences will typically be determined using standard dye terminator sequencing in conjunction with automated
5 sequencers (e.g., ABI sequencers and the like).

Several fragments of various sizes may serve as substrates for the sequencing reactions. This is not a problem since the sequencing reaction proceeds from a fixed point as defined by a specific primer sequence. Typically,
10 approximately 200 nucleotides of sequence were obtained for each trapped transcript. For the PCR fragments that are shorter than this, the sequencing reaction simply 'falls off' the end. Sequences further 3' were then covered by the longer fragments amplified during PCR. One problem is
15 presented by the anchor sequences 'S' derived from the RS primer. When these are encountered during the sequencing of smaller fragments, they register as anomalous dye signals on the sequencing gels. To circumvent this potential problem, a restriction enzyme recognition site is included in the S
20 sequence. Digestion of the double stranded PCR products with this enzyme prior to sequencing eliminates the heterologous S sequences.

5.2.3. Identifying the Tagged Genes by Chromosomal Location

25 Any individually tagged gene may also be identified by PCR using chromosomal DNA as the template. To find an individual clone of interest in the Library arrayed as described above, genomic DNA is isolated from the pooled
30 clones of ES cells as presented in Figure 3. One primer for the PCR is anchored in the gene trap vector, e.g., a puro exon-specific oligonucleotide. The other primer is located in the genomic DNA of interest. This genomic DNA primer may consist of either (1) DNA sequence that corresponds to the
35 coding region of the gene of interest, or (2) DNA sequence from the locus of the gene of interest. In the first case, the only way that the two primers used may be juxtaposed to

give a positive PCR results (e.g., the correct size double-stranded DNA product) is if the gene trap vector has inserted into the gene of interest. Additionally, degenerate primers may be used, to identify and isolate related genes of

5 interest. In the second case, the only way that the two primers used may be juxtaposed to provide the desired PCR result is if the gene trap vector has inserted into the region of interest that contains the primer for the known marker.

10 For example, if one wishes to obtain ES cell clones from the library that contain mutated genes located in a certain chromosomal position, PCR primers are designed that correspond to the puro gene (the puro-anchored primer) and a primer that corresponds to a marker known to be located in
15 the region of interest. Several different combinations of marker primers and primers that are located in the region of interest may also be used to obtain optimum results. In this manner, the mutated genes are identified by virtue of their location relative to sets of known markers. Genes in a
20 particular chromosomal region of interest could therefore be identified. The marker primers could also be designed correspond to sequences of known genes in order to screen for mutations in particular genes by PCR on genomic DNA templates. While this method is likely to be less
25 informative than the RT-PCR strategy described below, this technique would be useful as a alternative strategy to identify mutations in known genes. In addition, primers that correspond to sequence of known genes could be used in PCR reactions with marker-specific primers in order to identify
30 ES cell clones that contain mutations in genes proximal to the known genes. The sensitivity of detection is adequate to find such events when positive clones are subsequently identified as described below in the RT-PCR strategy.

35 5.3. A Sequence Database Identifies Genes Mutated in the Library.

Using the procedures described above, approximately 200

to about 600 bases of sequence from the cellular exons appended to the selectable marker exon (e.g., *puro* exon in VICTR vectors) may be identified. These sequences provide a means to identify and catalogue the genes mutated in each 5 clone of the Library. Such a database provides both an index for the presently disclosed libraries, and a resource for discovering novel genes. Alternatively, various comparisons can be made between the Library database sequences and any other sequence database as would be familiar to those 10 practiced in the art.

The novel utility of the Library lies in the ability for a person to search the Library database for a gene of interest based upon some knowledge of the nucleic acid or amino acid sequence. Once a sequence is identified, the 15 specific clone in the Library can be accessed and used to study gene function. This is accomplished by studying the effects of the mutation both *in vitro* and *in vivo*. For example, cell culture systems and animal models (i.e., transgenic animals) may be directly generated from the cells 20 found in the Library as will be familiar to those practiced in the art.

Additionally, the sequence information may be used to generate a highly specific probe for isolating both genomic clones from existing data bases, as well as a full length 25 cDNA. Additionally, the probe may be used to isolate the homologous gene from sufficiently related species, including humans. Once isolated, the gene may be over expressed, or used to generate a targeted knock-out vector that may be used to generate cells and animals that are homozygous for the 30 mutation of interest. Such animals and cells are deemed to be particularly useful as disease models (i.e., cancer, genetic abnormalities, AIDS, etc.), for developmental study, to assay for toxin susceptibility or the efficacy of therapeutic agents, and as hosts for gene delivery and 35 therapy experiments (e.g., experiments designed to correct a specific genetic defect *in vivo*).

5.4. Accessing Clones in the Library by a Pooling and Screening Procedure.

An alternative method of accessing individual clones is by searching the Library database for sequences in order to isolate a clone of interest from pools of library clones. The Library may be arrayed either as single clones, each with different insertions, or as sets of pooled clones. That is, as many clones as will represent insertions into essentially every gene in the genome are grown in sets of a defined number. For example, 100,000 clones can be arrayed in 2,000 sets of 50 clones. This can be accomplished by titrating the number of VICTR retroviral particles added to each well of 96-well tissue culture plates. Two thousand clones will fit on approximately 20 such plates. The number of clones may be dictated by the estimated number of genes in the genome of the cells being used. For example, there are approximately 100,000 genes in the genome of mouse ES cells. Therefore, a Library of mutations in essentially every gene in the mouse genome may be arrayed onto 20 96-well plates.

To find an individual clone of interest in the Library arrayed in this manner, reverse transcription-polymerase chain reactions (RT-PCR) are performed on mRNA isolated from pooled clones as presented in Figure 4. One primer for RT-PCR is anchored in the gene trap vector, i.e. a *puro* exon-specific oligonucleotide. The other primer is located in the cDNA sequence of a gene of interest. The only way that these two sequences can be juxtaposed to give a positive RT-PCR result (i.e. double stranded DNA fragment visible by agarose gel electrophoresis, as will be familiar to anyone practiced in the art) is by being present in a transcript from a gene trap event occurring in the gene of interest.

For example, if one wishes to obtain an ES cell clone with a mutation in the *p53* gene, PCR primers are designed that correspond to the *puro* and *p53* genes. If a VICTR trapping vector integrates into the *p53* locus and results in the formation of a fusion mRNA, this mRNA may be detected by RT-PCR using these specifically designed primer pairs. The

sensitivity of detection is adequate to find such an event when positive cells are mixed with a large background of negative cells. The individual positive clones are subsequently identified by first locating the pool of 50 clones in which it resides. This process is described in Figure 5. The positive pool, once identified, is subsequently plated at limiting dilution (approximately 0.3 cells/well) such that individual clones may be isolated. To find the one positive event in 50 clones represented by this pool, individual clones are isolated and arrayed on a 96-well plate. By pooling in columns and rows, the positive well containing the positive clone can be identified with relatively few RT-PCR reactions.

In addition to RT-PCR, the pools may be screened by hybridization techniques (see generally Sambrook et al., 1989, Molecular Cloning: H Laboratory Manual 2nd edition, Cold Spring Harbor Press, Cold Spring Harbor, and Current Protocols in Molecular Biology, 1995, Ausubel et al. eds., John Wiley and Sons). Specific PCR fragments are generated from the mutated genes essentially as described above for the sequencing protocols of the individual clones (first-strand synthesis using RT primed by a random or oligo dT primer that is appended to a specific primer binding site). The gene trap DNA is amplified from the primer sets in the puro gene and the specific sequences appended to the RT primer. If this were done with pools, the resulting pooled set of amplified DNA fragments could be arrayed on membranes and probed by radioactive, or chemically or enzymatically labeled, hybridization probes specific for a gene of interest. A positive radioactive result indicates that the gene of interest has been mutated in one of the clones of the positively-labeled pool. The individual positive clone is subsequently identified by PCR or hybridization essentially as outlined above.

Alternatively, a similar strategy may be used to identify the clone of interest from multiple plates, or any scheme where a two or three dimensional array (e.g., columns

and rows) of individual clones are pooled by row or by column. For example, 96 well plates of individual clones may be arranged adjacent to each other to provide a larger (or virtual/figurative) two dimensional grid (e.g., four plates
5 may be arranged to provide a net 16x24 grid), and the various rows and columns of the larger grid may be pooled to achieve substantially the same result.

Similarly, plates may simply be stacked, literally or figuratively, or arranged into a larger grid and stacked to
10 provide three dimensional arrays of individual clones. Representative pools from all three planes of the three dimensional grid may then be analyzed, and the three positive pools/planes may be aligned to identify the desired clone. For example, ten 96 well plates may be screened by pooling
15 the respective rows and columns from each plate (a total of 20 pools) as well as pooling all of the clones on each specific plate (10 additional pools). Using this method, one may effectively screen 960 clones by performing PCR on only 30 pooled samples.

20 The example provided below is merely illustrative of the subject invention. Given the level of skill in the art, one may be expected to modify any of the above or following disclosure to produce insubstantial differences from the specifically described features of the present invention. As
25 such, the following example is provided solely by way of illustration and is not included for the purpose of limiting the invention in any way whatsoever.

6.0. EXAMPLES

30 6.1. Use of VICTR Series Vectors to Construct a Mouse ES cell Gene Trap Library

VICTR 3 was used to gather a set of gene trap clones. A plasmid containing the VICTR 3 cassette was constructed by conventional cloning techniques and designed to employ the
35 features described above. Namely, the cassette contained a PGK promoter directing transcription of an exon that encodes the puro marker and ends in a canonical splice donor

sequence. At the end of the puromycin exon, sequences were added as described that allow for the annealing of two nested PCR and sequencing primers. The vector backbone was based on pBluescript KS+ from Stratagene Corporation.

5 The plasmid construct linearized by digestion with Sca I which cuts at a unique site in the plasmid backbone. The plasmid was then transfected into the mouse ES cell line AB2.2 by electroporation using a BioRad Genepulser apparatus. After the cells were allowed to recover, gene trap clones
10 were selected by adding puromycin to the medium at a final concentration of 3 μ g/mL. Positive clones were allowed to grow under selection for approximately 10 days before being removed and cultured separately for storage and to determine the sequence of the disrupted gene.

15 Total RNA was isolated from an aliquot of cells from each of 18 gene trap clones chosen for study. Five micrograms of this RNA was used in a first strand cDNA synthesis reaction using the "RS" primer. This primer has unique sequences (for subsequent PCR) on its 5' end and nine
20 random nucleotides or nine T (thymidine) residues on its 3' end. Reaction products from the first strand synthesis were added directly to a PCR with outer primers specific for the engineered sequences of puromycin and the "RS" primer. After amplification, an aliquot of reaction products were subject
25 to a second round of amplification using primers internal, or nested, relative to the first set of PCR primers. This second amplification provided more reaction product for sequencing and also provided increased specificity for the specifically gene trapped DNA.

30 The products of the nested PCR were visualized by agarose gel electrophoresis, and seventeen of the eighteen clones provided at least one band that was visible on the gel with ethidium bromide staining. Most gave only a single band which is an advantage in that a single band is generally
35 easier to sequence. The PCR products were sequenced directly after excess PCR primers and nucleotides were removed by filtration in a spin column (Centricon-100, Amicon). DNA was

added directly to dye terminator sequencing reactions (purchased from ABI) using the standard M13 forward primer a region for which was built into the end of the *puro* exon in all of the PCR fragments. Thirteen of the seventeen clones
5 that gave a band after the PCR provided readable sequence. The minimum number of readable nucleotides was 207 and some of the clones provided over 500 nucleotides of useful sequence.

Sample data from this set of clones is presented in
10 Figure 6. Only a portion of sequence (nucleotide or putative amino acid) for 9 Library clones obtained by the methods described in this invention are presented. Under each sequence fragment in the figure is aligned a homologous
15 alignment search tool) search algorithm (Altschul et al., 1990, J. Mol. Biol. 215:403-410).

In addition to known sequences, many new genes were also identified. Each of these sequences is labeled "OST" for "Omnibank Sequence Tags." OMNIBANK™ shall be the trademark
20 name for the Libraries generated using the disclosed technology.

These data demonstrate that the VICTR series vectors may efficiently trap genes, and that the procedures used to obtain sequence are reliable. With simple optimization of
25 each step, it is presently possible to mutate every gene in a given population of cells, and obtain sequence from each of these mutated genes. The sample data provided in this example represents a small fraction of an entire Library. By simply performing the same procedures on a larger scale (with
30 automation) a Library may be constructed that collectively comprises and indexes mutations in essentially every gene in the genome of the target cell.

Additional studies have used both VICTR 3 and VICTR 20. Like VICTR 3, VICTR 20 is exemplary of a family of vectors
35 that incorporate two main functional units: a sequence acquisition component having a strong promoter element (phosphoglycerate kinase 1) active in ES cells that is fused

to the puromycin resistance gene coding sequence which lacks a polyadenylation sequence but is followed by a synthetic consensus splice donor sequence (PGKpuroSD); and 2) a mutagenic component that incorporates a splice acceptor sequence fused to a selectable, colorimetric marker gene and followed by a polyadenylation sequence (for example, SA β geopA or SAIRES β geopA). Also like VICTR 3, stop codons have been engineered into all three reading frames in the region between the 3' end of the selectable marker and the splice donor site. A diagrammatic description of structure and functions of VICTRs 3 and 20 is provided in Figure 7.

When VICTRs 3 and 20 were used in the commercial scale application of the presently disclosed invention, over 3,000 mutagenized ES cell clones were rapidly engineered and obtained. Sequence analysis obtained from these clones has identified a wide variety of both previously identified and novel sequences. A representative sampling of previously known genes that were identified using the presently described methods is provided in Figure 8. The power of the presently described invention as a genomics resource becomes apparent when one considers that the genes listed in Figure 8 were obtained and identified in less than a year whereas the references associated with the identification of the known genes span a period of roughly two decades. More importantly, the majority of the sequences thus far identified are novel, and, because of the functional aspects of the presently described ES cell system, the cellular and developmental functions of these novel sequences can be rapidly established.

30

7.0. Reference to Microorganism Deposits

The following plasmids have been deposited at the American Type Culture Collection (ATCC), Rockville, MD, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and Regulations thereunder (Budapest Treaty) and are thus maintained and made available according

to the terms of the Budapest Treaty. Availability of such plasmids is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The deposited cultures have been assigned the indicated ATCC deposit numbers:

	<u>Plasmid</u>	<u>ATCC No.</u>
	plex	97748
10	pExonII	97749
	ppuro7	97750
	ppuro5	97751
	ppuro11	97752
	ppuro10	97753

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the above-described modes for carrying out the invention which are obvious to those skilled in the field of molecular biology or related fields are intended to be within the scope of the following claims.

30

35

MICROORGANISMS	
Optional Sheet in connection with the microorganism referred to on page <u>40</u> , lines <u>5-25</u> of the description *	
A. IDENTIFICATION OF DEPOSIT * Further deposits are identified on an additional sheet *	
Name of depositary institution * American Type Culture Collection	
Address of depositary institution (including postal code and country) * 12301 Parklawn Drive Rockville, MD 20852 US	
Date of deposit * <u>October 9, 1996</u> Accession Number * <u>97748</u>	
B. ADDITIONAL INDICATIONS * (leave blank if not applicable). This information is continued on a separate attached sheet	
C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (if the indications are not all designated States)	
D. SEPARATE FURNISHING OF INDICATIONS * (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")	
E. <input type="checkbox"/> This sheet was received with the International application when filed (to be checked by the receiving Office)	
<div style="text-align: right;">_____ (Authorized Officer)</div>	
<input type="checkbox"/> The date of receipt (from the applicant) by the International Bureau *	
was _____ (Authorized Officer)	

Form PCT/RO/134 (January 1981)

International Application No: PCT/ /

Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parklawn Drive
Rockville, MD 20852
USAccession No.Date of Deposit

97749

October 9, 1996

97750

October 9, 1996

97751

October 9, 1996

97752

October 9, 1996

97753

October 9, 1996

CLAIMSWhat is claimed is:

1. A library of cultured eucaryotic cells made by a process comprising the steps of:
 - 5 a) treating a first group of cells to stably integrate a first vector that mediates the splicing of a foreign exon internal to a cellular transcript;
 - b) treating a second group of cells to stably integrate a second vector that mediates the splicing of a foreign exon
10 5' to an exon of a cellular transcript; and
 - c) selecting for transduced cells that express the products encoded by the foreign exons.
2. A library according to claim 1 wherein said treating
15 is transfection.
3. A library according to claim 1 wherein said treating is by infection.
- 20 4. A library according to claim 1 wherein said treating is by retrotransposition.
5. A library according to any one of claims 1 through 4 wherein said cells are animal cells.
25
6. A library according to claim 5 wherein said animal is mammalian.
7. A library according to claim 6 wherein said cells
30 are rodent cells.
8. The use of a mutated cell from a library according to claim 6 to generate a non-human transgenic animal.
- 35 9. A vector for replacing the 3' end of an animal cell transcript with a foreign exon, comprising:
 - a) a selectable marker;

- 5 b) a splice acceptor site operatively positioned 5' to the initiation codon of said selectable marker;
- c) a polyadenylation site operatively positioned 3' to said selectable marker;
- d) said vector not comprising a promoter element operatively positioned 5' of the coding region of said selectable marker; and
- 10 e) said vector not comprising a splice donor sequence operatively positioned between the 3' end of the coding region of said selectable marker and said polyadenylation site.

10. A vector for inserting foreign mutagenic polynucleotide sequence internal to animal cell transcripts, comprising:

- 15 a) a foreign exon;
- b) a splice acceptor sequence operatively positioned 5' to the foreign exon;
- 20 c) a splice donor site operatively positioned 3' to said foreign exon;
- d) a sequence comprising a nested set of stop codons in each of the three reading frames located between the 3' end of said foreign exon and said splice donor site;
- 25 e) said vector not comprising a polyadenylation site operatively positioned 3' to said foreign exon; and
- f) said vector not comprising a promoter element operatively positioned 5' to the coding region of said foreign exon.

30

11. A vector for attaching a foreign exon upstream from the 3' end of an animal cell transcript, comprising:

- 35 a) a selectable marker;
- b) a promoter element operatively positioned 5' to said selectable marker;
- c) a splice donor site operatively positioned 3' to said selectable marker; and

- d) said vector not comprising a transcription terminator or polyadenylation site operatively positioned relative to the coding region of said selectable marker; and
- 5 e) said vector not comprising a splice acceptor site operatively positioned between said promoter element and the initiation codon of said selectable marker.

10 12. A vector according to claim 11 wherein said vector additionally comprises a foreign mutagenic polynucleotide sequence located upstream from said promoter.

13. A vector according to claim 12 wherein said vector
15 additionally comprises a splice acceptor operatively positioned upstream from said foreign mutagenic polynucleotide sequence.

14. A vector according to claim 13 wherein said foreign
20 mutagenic polynucleotide sequence comprises a polyadenylation site.

15. A vector according to claim 14, wherein said foreign mutagenic polynucleotide sequence additionally
25 comprises stop codons in all three reading frames.

16. A vector according to claim 12 in which a first recombinase recognition sequence is present upstream from said promoter and a second recombinase recognition sequence
30 is present downstream from said promoter.

17. A vector according to any one of claims 9, 10, or 11 wherein said vector is a viral vector.

35 18. A vector according to claim 17 wherein said viral vector is a retroviral vector.

19. The use of a vector according to claim 9 to produce a library of mutated animal cells.

20. The use of a vector according to claim 10 to produce mutated animal cells.

21. The use of a vector according to claim 11 to produce mutated animal cells.

22. The use of a vector according to claim 11 to effect homologous recombination in an animal cell.

23. A stably transduced animal cell that incorporates a vector according to claim 16.

15

24. A method of deleting a region of vector DNA from a cell according to claim 23, comprising:

- a) providing a recombinase activity to the cell; and
- b) selecting for cells that lack the desired region of vector DNA.

20

25. A method of adding a region of DNA to a cell according to claim 23, comprising:

- a) introducing the DNA to be added into the cell;
- a) providing a recombinase activity to the cell; and
- b) selecting for cells that incorporate the added DNA.

25

26. A method of effecting the inducible expression of a desired gene, comprising:

- a) providing a cell according to claim 23 with a recombinase gene that is expressed by an inducible promoter; and
- b) inducing said inducible promoter.

30

27. A method of gene discovery comprising:

- a) adding a foreign polynucleotide to a population of target cells such that the foreign

35

polynucleotide is inserted throughout the genomes of the target cells; and

b) activating control elements encoded by the foreign polynucleotides that activate or repress the expression of target cell genes that flank the integrated foreign polynucleotides, and identifying the regions of the target cell genome into which the foreign polynucleotides have integrated.

28. A library of cultured animal cells that stably integrate vectors according to claims 10 or 11.

15

20

25

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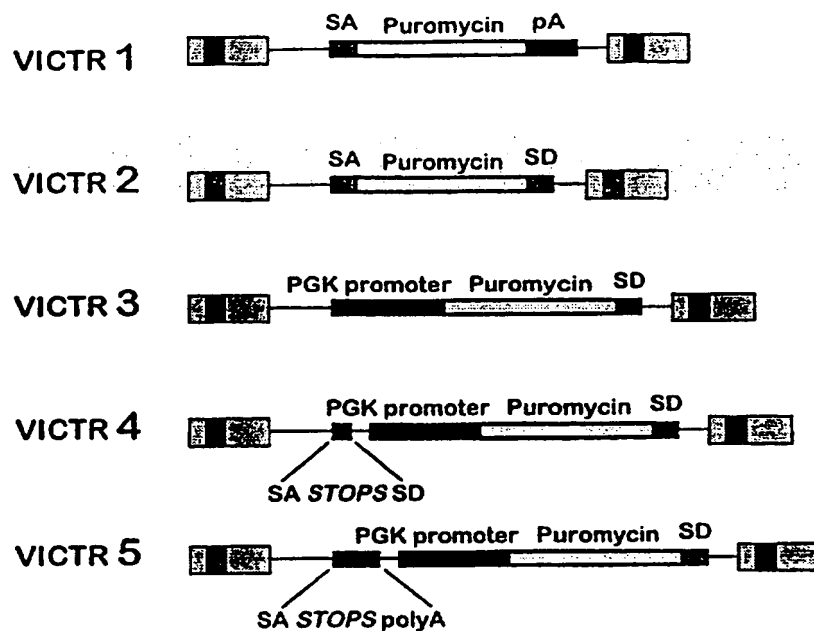


Figure 1

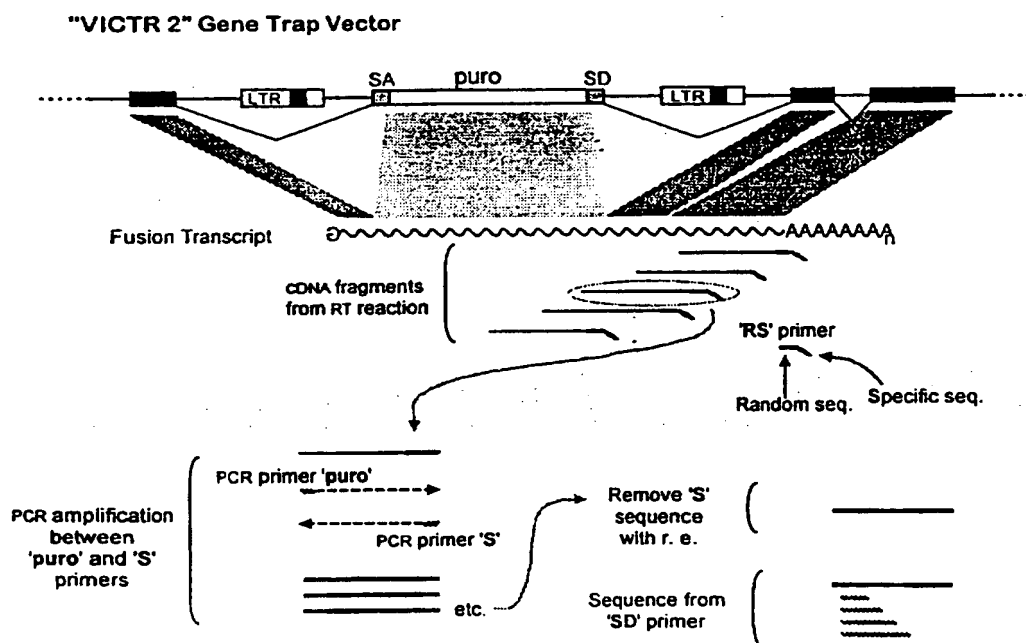


Figure 2

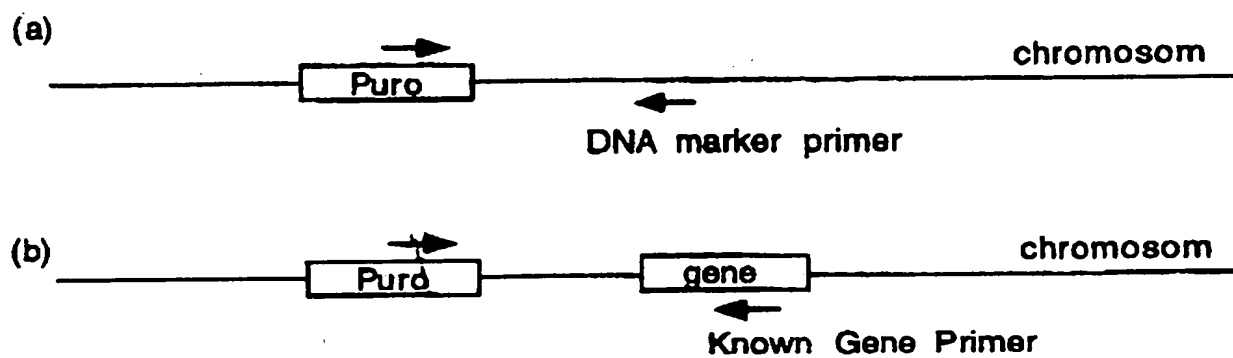
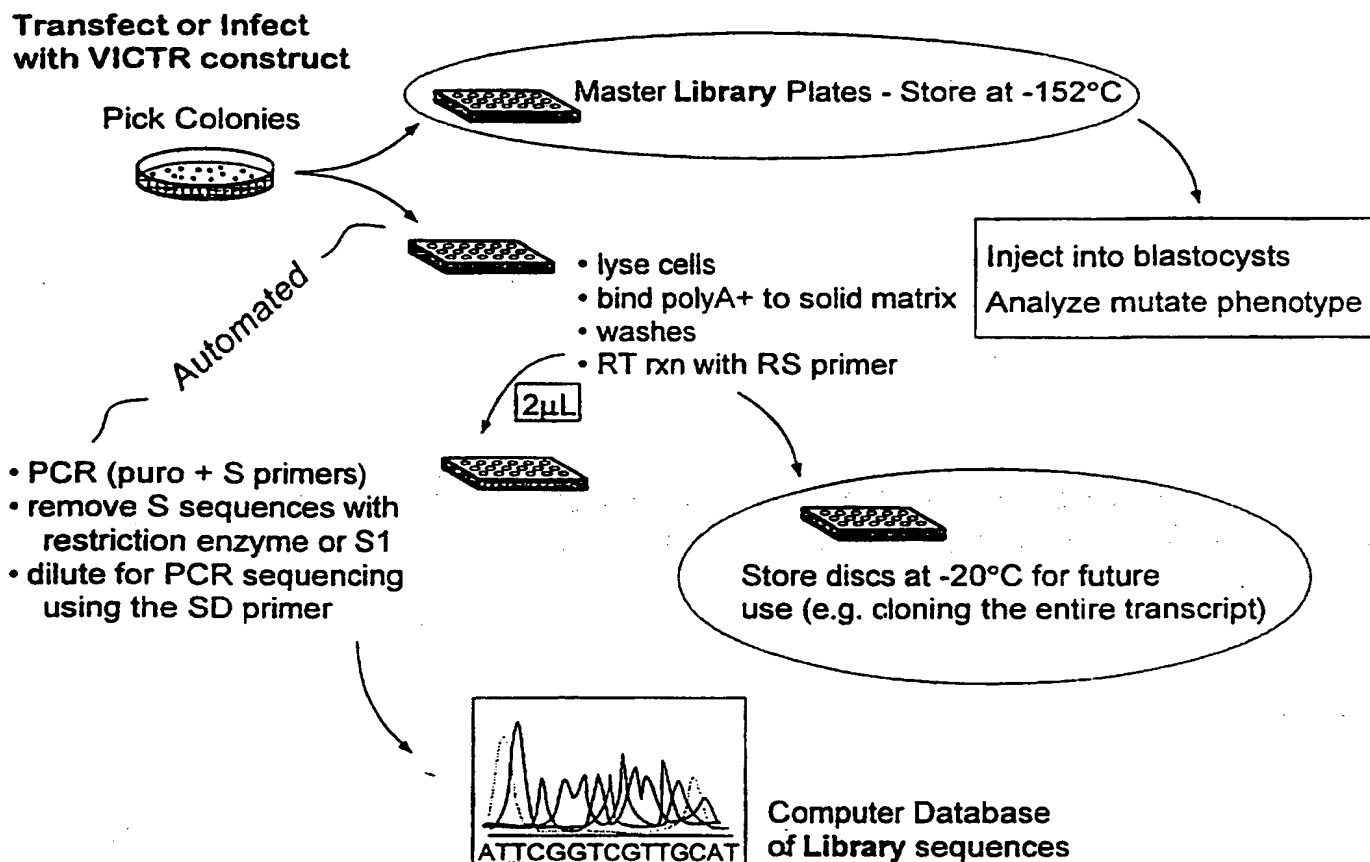
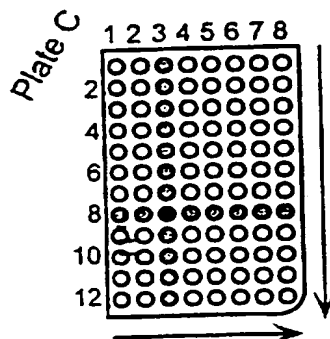


Figure 3

**Figure 4**

Identify Positive Pool

To screen all mouse genes (~100,000) with 5-fold redundancy would require about 50 plates of 96-wells (at 100 clones/well).



① Pool columns

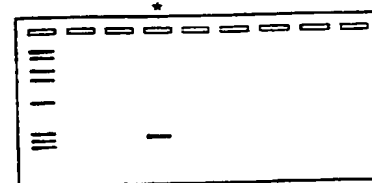
(100 clones/well)(12 wells) = 1200 clones per rxn.
(50 plates)(8 columns/plate) = 400 initial rxns.

Plate C, column 3 was positive.

② Pool positive rows only

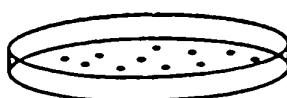
8 rxns per positive plate

Row 8 was positive.

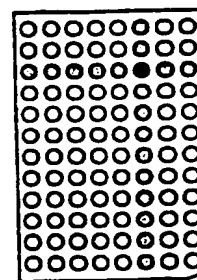


Identify Positive Clone

The pool on plate C, column 3, row 8 is thawed and plated as single clones:



① Pick 96 colonies into two 96-well plates



② Pool columns (16) and rows (24) for 40 rxns to identify positive clone.

Figure 5

OST1:	248	TTTATATAATATTAAATTTGTTTACTGGGGTATATATGTGTGAAGAGGACTTCT	302
rat GABA rho3:	1547	TTTACATAATATTAAATTTGTTTACTGGGGTATATATGTGTGAAGAGGACTTTT	1601
OST2:	56	ACCGTTGCGGAGGCTCAGGTTTCTCAGATAGTACATCAGGTGTCATCGNTGTCAGAAGGT	115
mouse TCR-ATF1:	75	ACCGTTGCGGGGCTCAGGTTTCTCAGATAGTACATCAGGTGTCATCGTTATCAGAAAGT	134
OST3:	58	GIGMHAGLHERDRKTVLELFXNCKVQVLIATSTLAWGVNFPAPHLVLIKGT EYDGTTRR	237
Yeast ORF G9365:	1430	GIGLHHAGLVQKDRSISHQLFQNKIQIL IATSTLAWGVNLPAPHLVLIKGTQFFDAKIEG	1489
OST4:	137	GCGCAGAAGTGGTNCCTGGAANTTTNTCCGCCNCCATCCAGTCTATTAAATGTTGACNGGA	196
seq. from US	166	GCGCAGAAGTGGTCCCTGCAACTTTATCCGCCCTCCATCCAGTCTATTAAATGTTGCCGGGA	225
patent 5470724:			
OST5:	108	TCWIRLGT*RXVGASLEYEYIRAS	179
mouse wnt-5A		TCW++L R VG +L+ +Y A+	
protein precursor:	250	TCWLQLADFRKVGDALKEKYDSAA	273
OST6:	78	CTTATATGGCTACGGCGGCTTCAACATCTCCATTACACCCAACACAGCGTGTCCAGGCT	137
human prolyl			
endopeptidase:	1407	CTTATATGGCTATGGCGGCTTCAACATATCCATCACACCCAACACAGTGTTCAGGCT	1466
OST7:	109	AAAGCATGTAGCAGTTGTAGGACACACTAGACGAGAGCACCAGATCTCATTTGTGGGTGGT	168
mouse			
45S pre rRNA:	1604	AAAGCATGTAGCAGTTGTAGGACACACTAGACGAGAGCACCAGATCTCATTTGTGGGTGGT	1663
OST8:	161	TGGATGCAGNCTACCACCTGTGTGGCTGCCCTATTMTACCTCAGTGCTCAGTTCTGGAAG	220
rat MAL:	306	TGGATGCAGCCTACCACCTGTGTGGCTGCCCTGTMTTACCTCAGTGCTCAGTCCTGGAAG	365
OST9:	103	ACCTGATTGTTATCCGTGGCCTGCAGAAGTCCAGAAAATACAGACCAAAGTCAACCAGTA	162
mouse malic enzyme:	1666	ACCTGATTGTTATCCGTGGCCTGCAGAAGTCCAGAAAATACAGACCAAAGTCAACCAGTA	1725

Figure 6

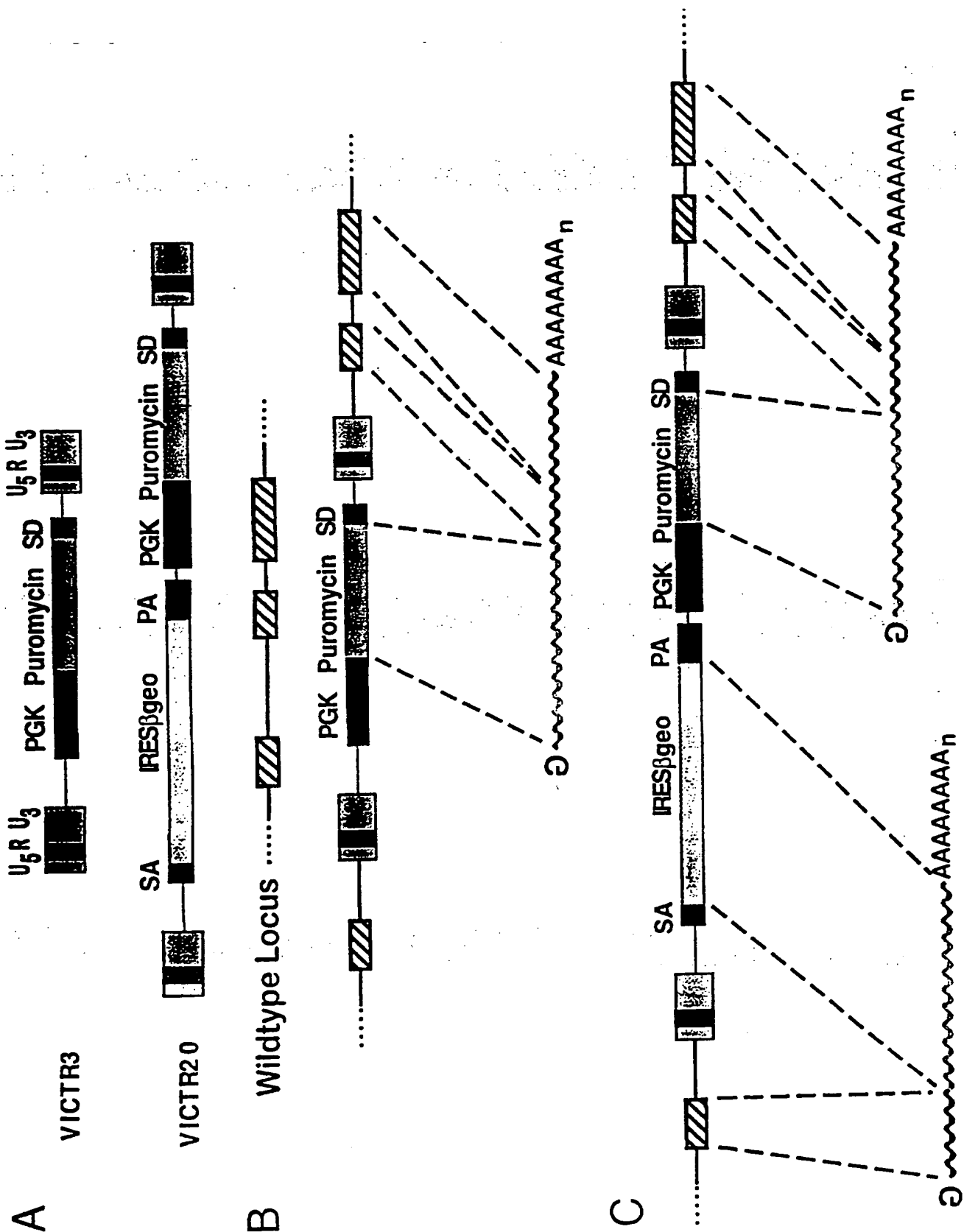


Figure 7

Figure 8

The following table includes 586 OSTs. OSTs with hit into prdcom and Genbank patented sequences have been removed as well as sequence with repetitive elements hits

Genbank	Accession	Value	ID	Sequence Description
U274	gb U09445	5.0e-111	901	Mus musculus m43102 r1 Soares mouse clone 115187 5'
U275	gb U09446	5.0e-11	95A	Mus musculus mouse mRNA for retinal cyclic-GMP phosphodiesterase
OST22	gb D88454	5.9e-48	83A	Gemea-subunit (GMP-PDE) (SC 3.1.4.17)
OST25	gb U28168	1.0e-42	87A	Mus musculus mouse mRNA complete cds
OST30	gb AA048968	1.9e-173	98A	Mus musculus m50b06.r1 Soares mouse embryo NMME13.5 1A.5 Mus musculus cDNA clone 479507 5'
OST36	gb U29016	7.5e-71	90A	Mus musculus mouse mRNA for aqualene synthase
OST38	gb X53732	3.0e-106	95A	Mus musculus M.musculus T cell receptor alpha chain variable region (V-alpha)
OST41	gb J00360	1.8e-70	101	Mus musculus mouse alpha-amylase-2 gene: pancreatic mra
OST42	gb M33190	4.0e-34	62A	Rattus norvegicus Rat cytochrome P450 II A3 (CYP2A3) gene, complete cds
OST45	gb AA003309	1.4e-145	99A	Mus musculus m47d10.r1 Soares mouse embryo NMME13.5 1A.5 Mus musculus cDNA clone 426931 5'
OST53	gb D86214	1.5e-45	66A	Mus musculus mouse mouse: Musculus domesticus Postnatal (0 day) Brain mRNA for Ca2+ dependent activator protein for secretion, complete cds
OST56	gb AA189233	2.6e-37	91A	Mus musculus mu52c11.r1 Soares mouse lymph node NLMH Mus musculus cDNA clone 643028 5' similar to TR:G294850 G294850 ALPHA-MUSCLE ACTIN
OST74	gb Y00169	7.5e-112	89A	Rattus norvegicus Rat Th-4 gene for fibroblast tropomyosin 4
OST75	gb Z72384	1.0e-126	95A	Mus musculus M.musculus 1g1-Va2(70/3) gene
OST86	gb AA390122	1.7e-31	88A	Mus musculus mu6205.r1 Soares mouse lymph node NLMH Mus musculus cDNA clone 62485 5' similar to life Tech mouse embryo B50C 106640 5'
OST95	gb AA104745	1.8e-178	96A	Mus musculus mu55573.5 similar to SH-XA15 SCIRPO 089313 HYPOTHETICAL 37 7 KD PROTEIN clone 11.06 2M CHN1
OST98	gb U13306	7.3e-40	88A	Rattus sp. EST110153 Rattus sp. cDNA 5' end
OST117	gb AA156426	4.0e-111	97A	Homo sapiens a151b07 s1 Soares pregnant uterus HUMPU Homo sapiens cDNA clone 505429 3' similar to TR:G622498 G622498 CLEAVAGE STIMULATION FACTOR 77KDa SUBUNIT
OST118	gb U8764	8.6e-154	84A	Homo sapiens human mRNA for KIAA0242 gene, partial cds
OST119	gb D87077	2.0e-145	92A	Homo sapiens human mRNA for KIAA0240 gene, partial cds
OST121	gb D28482	3.1e-161	83A	Homo sapiens human scf2 mRNA for RNA binding protein SCR2, complete cds
OST133	gb AA14106	1.2e-52	71A	Homo sapiens tigg6h09.r1 Stratyene HeLa cell s1 937216 Homo sapiens cDNA clone 563201 5'
OST154	gb AA107943	4.0e-128	82A	Mus musculus mu59c06.r1 Life Tech mouse embryo 10 5dpc 18665016 Mus musculus cDNA clone 556906 5' similar to gb J05277 Mouse luxokinase mRNA, complete cds (MOUSE)
OST178	gb X65300	8.1e-143	92A	Ribophorin I
OST193	gb C06148	4.8e-107	84A	Homo sapiens similar to glutamyl- (NH2) pyruvate EST106073 Rattus sp. cDNA 5' end similar to Spvaf1
OST213	gb U12166	4.8e-38	86A	Mus musculus m401b09.r1 Soares mouse embryo NMME13.5 1A.5 Mus musculus cDNA clone 441209 5'
OST216	gb AA009122	1.8e-81	79A	Mus musculus mouse 4 5S rRNA gene
OST268	gb M12658	1.2e-91	93A	Mus musculus m47d10.r1 Soares mouse embryo NMME13.5 1A.5 Mus musculus cDNA clone 438764 5'
OST280	gb AA058245	1.5e-141	94A	

Figure 8 cont'd.

[illegible]

Figure 8 cont'd.

OST1006	gb U087077	7.7e-112	881	(CA31.1) mRNA, complete cds
OST1105	gb U044423	1.0e-66	861	homo sapiens human mRNA for KIAA0310 gene, partial cds
OST1116	gb U042249	1.1e-76	801	homo sapiens 122801.01.1 Soares mouse
OST1137	gb U043485	1.2e-89	941	sapiens cDNA clone 31647.3
OST1145	gb U080040	1.4e-128	911	Mus musculus H. musculus cDNA
OST1152	gb U079970	6.4e-103	101	Rattus norvegicus Rat. cDNA
OST1175	gb U010124	2.1e-63	951	Mus musculus m42001.11.1 Soares mouse
OST1165	gb U054649	1.9e-184	971	INTERLON-INDUCIBLE PROTEIN 1-BU
OST1179	gb U080986	8.5e-84	911	APOLIPROTEIN C-II, precursor
OST1186	gb U089840	2.8e-70	961	Mus musculus m42001.11.1 Soares mouse
OST1192	gb U082490	5.1e-127	961	complete cds
OST1207	gb U0663763	1.1e-56	861	Mus musculus m42001.11.1 Soares mouse
OST1223	gb U0802931	1.5e-189	931	OST1223.5
OST1226	gb U073753	7.5e-279	951	Mus musculus m42001.11.1 Soares mouse
OST1233	gb U098701	6.1e-91	981	phosphatase 2A B-alpha regulatory subunit mRNA, partial cds
OST1234	gb U085904	1.1e-180	981	Mus musculus m42001.11.1 Soares mouse
OST1241	gb U098850	4.8e-184	971	complete cds
OST1247	gb U08051266	4.7e-126	971	Mus musculus m42001.11.1 Soares mouse
OST1265	gb U096973	6.8e-183	961	OST1265.5
OST1267	gb U084271	1.6e-12	861	homo sapiens human
OST1269	gb U019977	4.0e-130	841	preprocarboxypeptidase A3 (prCPA3) mRNA, complete cds
OST1274	gb U014614	2.1e-119	851	Rattus norvegicus Rat. mitochondrial
OST1294	gb U030583	6.8e-69	951	beta-subunit mRNA, complete cds
OST1339	gb U097190	3.1e-116	831	Rattus norvegicus Rat. cDNA
OST1441	gb U089611	7.8e-142	931	complete cds
OST1454	gb U074401	2.2e-64	851	Mus musculus m42001.11.1 Soares mouse
OST1459	gb U062672	5.1e-35	971	homo sapiens human
OST1463	gb U062650	1.1e-109	971	UNIQUELY-CONCATENATING ENZYME E2-17 RD

adhesion molecule 1
Mus musculus H. musculus Cank-2 mRNA
kinase II beta subunit
homo sapiens human fetal brain cDNA
5'-end cDNA-149C01
Mus musculus m42001.11.1 Soares mouse
embryo NMEL13.5 14.5 Mus musculus cDNA
clone 374002.5
Mus musculus m42001.11.1 Soares mouse
p10NF19.5 Mus musculus cDNA clone
311148.5
Mus musculus m42001.11.1 Soares mouse
embryo NMEL13.5 14.5 Mus musculus cDNA
clone 420325.5
homo sapiens human placenta cDNA
5'-end cDNA-560009
homo sapiens y190103.11.1 Soares mouse
cDNA clone 166525.5
Mus musculus mouse guanylate
cyclase/atrial natriuretic factor
receptor mRNA, complete cds
Mus musculus H. musculus inhibitor of
MyoD family-b (1-mf) mRNA, complete
cds
synthetic construct Synthetic
NADH:cytochrome c reductase fusion
protein mRNA, complete cds
Mus musculus m42001.11.1 Soares mouse
p10NF19.5 Mus musculus cDNA clone
311156.5
Mus musculus m42001.11.1 Soares mouse
p10NF19.5 Mus musculus cDNA clone
493155.5 similar to gb|U05093.605
RIBOSOMAL PROTEIN L18A (HUMAN)
homo sapiens y059005.11.1 Soares mouse
cDNA clone 78873.5 Mus musculus integral
membrane protein 1 (Ital) mRNA,
complete cds
Mus musculus H. musculus core-binding
factor mRNA sequence
Mus musculus m42001.11.1 Soares mouse
p10NF19.5 Mus musculus cDNA clone
334596.5 similar to PIN-310451 J10457
BOK nuclear protein - human
Rattus norvegicus Rattus norvegicus
CLP36 (clp36) mRNA, complete cds
Mus musculus m42001.11.1 Soares mouse
embryo NMEL13.5 14.5 Mus musculus cDNA
clone 407721.5 similar to
SW:1PVL:HOVIN:117900 (HUMAN)
MIRNID:PHNATAC
Mus musculus m42001.11.1 Soares mouse
mouse embryonic region Mus musculus
cDNA clone 533141.5
Rattus sp. EST110085 Rattus sp. cDNA
5'-end
Mus musculus H. musculus
Serine/threonine protein kinase DIX
mRNA, complete cds
homo sapiens y190103.11.1 Soares mouse
cDNA clone 324390.5
Mus musculus m42001.11.1 Soares mouse
p10NF19.5 Mus musculus cDNA clone
315182.5 similar to PIN-310451 J10457
hypothetical protein 14167.23 - yeast
Mus musculus H. musculus
lysosomal-associated
multisubunit protein (LAMP1)
mRNA, complete cds
Mus musculus m42001.11.1 Soares mouse
embryo NMEL13.5 14.5 Mus musculus cDNA
clone 479172.5
Mus musculus m42001.11.1 Soares mouse
embryo NMEL13.5 14.5 Mus musculus cDNA
clone 464783.5
Mus musculus m42001.11.1 Soares mouse
p10NF19.5 Mus musculus cDNA clone

Figure 8 cont'd.

OST2210	gb AA001277	6.5e-118	91%	Mus musculus m31308 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 478913.5. Similar to gb A007641 SH-100 CYTOCHROME C HEMITASK 11 KD HOMO sapiens (HUMAN)
OST2112	gb W01370	4.7e-118	96%	Mus musculus m31303 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 407837.5. Similar to gb K00558 TUBULIN ALPHA-1 CHAIN (HUMAN); gb M13441 Mouse alpha-tubulin 1:20 type M-alpha-6 mRNA, complete cds
OST2116	gb X16453	2.4e-66	86%	Rattus norvegicus R. norvegicus (Erythrina Doulay) 11-rv0107 mRNA
OST2126	gb W09867	1.8e-35	85%	Mus musculus m31302 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 425259.5
OST2134	gb W14001	5.2e-109	91%	Mus musculus m31301 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 315504.5. Similar to P11-35086 35086 4E-B1 protein (human)
OST2143	gb H91351	3.8e-40	79%	Homo sapiens y087007 r1 Homo sapiens CDNA clone 240780.5
OST2165	gb H64326	1.7e-144	91%	Mus musculus m35541 r1 Soares mouse clone 385724.5
OST2174	gb U05055	1.1e-67	91%	Mus musculus Mus musculus r3p1/4002 Interacting protein 14004 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 418711.5
OST2177	gb W99350	1.9e-122	86%	Mus musculus m31304 r1 Soares mouse clone 418711.5
OST2182	gb S79780	1.4e-55	91%	Mouse DP-3 protein regulating cell cycle transcription factor 1071/1271 (HUMAN); gb S79780.5
OST2188	gb W10048	2.3e-86	91%	Mus musculus m35700 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 315731.5. Similar to gb L02547 CLEAVAGE STIMULATION FACTOR, 50 KD SUBUNIT (HUMAN)
OST2191	gb U40854	5.4e-61	95%	Mus musculus Mus musculus SHC mRNA, complete cds
OST2198	gb T25302	3.1e-33	69%	Homo sapiens EST0398 Homo sapiens CDNA clone 8129-198
OST2218	gb W38136	4.1e-112	91%	Homo sapiens tcl1a11.51 Soares parathyroid tumor NM1P4 Homo sapiens CDNA clone 322268.3
OST2226	gb AAC49140	6.0e-153	91%	Mus musculus m31304 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 479430.5. Similar to SH-576 TORCA P13701 SYNAPTOMEVIN
OST2229	gb AA014563	2.8e-109	91%	Mus musculus m167c05 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 468584.5
OST2236	gb S63758	2.4e-90	96%	Mus sp. metallothionein-1 gene transcription activator (aice, L cells, mRNA, 509 nt)
OST2237	gb AA002285	1.7e-33	10%	Mus musculus m31306 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 426442.5. Similar to SH-Y081 YEAST P18326 HYPOPHYSICAL 14.1 KD PROTEIN IN PUDI-A001 INTERGENIC REGION, 111
OST2250	gb W17063	2.0e-137	96%	Mus musculus m31303 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 389114.5
OST2269	gb U16037	3.1e-119	94%	Mus musculus Mus musculus CREB transcription factor, novel spliced form, mRNA, partial cds
OST2271	gb U10005	9.9e-111	94%	Mus musculus Mus musculus tcl1 mRNA, complete cds
OST2275	gb W10226	1.7e-113	93%	Mus musculus m31304 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 349591.5. Similar to SH-C1C2 DUTIN P15526 CILONINE CUNNIEH PROTEIN P64
OST2285	gb W13930	1.2e-13	95%	Mus musculus m35408 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 313210.5. Similar to gb U13115 LACTOGLUTATHIONE LYASE (HUMAN)
OST2296	gb W10201	8.3e-85	96%	Mus musculus m31301 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 403221.5. Similar to
OST2297	gb P07714	7.8e-34	86%	SH-YASE SCHMO Q05790 HYPOPHYSICAL 23.7 KD PROTEIN C1366.18 IN CHROMOSOME 11. Homo sapiens H. sapiens partial CDNA sequence: clone c-70607
OST2307	gb X61399	7.0e-66	92%	Mus musculus Mouse 552 mRNA, full a novel protein
OST2321	gb AA100747	5.1e-85	78%	Homo sapiens y19104 r1 Stratagene colon (4937204) Homo sapiens CDNA clone 512033.5
OST2322	gb J00511	1.2e-81	92%	Rattus sp. Rat myadenylate deaminase (AMP deaminase) mRNA, complete cds
OST2346	gb AA000893	1.5e-95	94%	Mus musculus Mouse mRNA for myotomatin 3, complete cds
OST2347	gb U17653	2.5e-101	85%	Mus musculus Mouse mRNA for myotomatin 3, complete cds
OST2351	gb U10097	7.4e-73	85%	Mus musculus m31302 r1 Soares mouse CDNA clone 24692.5
OST2359	gb U10066	4.1e-113	91%	Mus musculus m31309 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 349316.5
OST2361	gb W18873	7.5e-58	95%	Mus musculus m30400 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 317927.5
OST2367	gb X97811	1.9e-104	85%	Rattus norvegicus R. norvegicus mRNA for carnitine/acetylcaritine carrier protein
OST2368	gb AA013837	6.1e-32	77%	Mus musculus m31306 r1 Soares mouse placenta (HBMPL) 5.14.5 Mus musculus CDNA clone 443434.5
OST2379	gb U10911	1.2e-105	91%	Homo sapiens Homo sapiens splicing factor (CCI.4) mRNA, complete cds
OST2380	gb W87091	9.5e-88	90%	Mus musculus m158110 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 418883.5
OST2381	gb AA080090	8.3e-126	96%	Mus musculus m31306 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 484187.5
OST2382	gb AA015380	5.1e-126	92%	Mus musculus m31302 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 460719.5. Similar to gb A11690 Mouse argininosuccinate lyase
OST2389	gb U05614	2.0e-59	91%	Mus musculus m31306 r1 Soares mouse complete cds
OST2395	gb AA122609	5.0e-118	10%	Mus musculus m31306 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 319003.5
OST2400	gb U24469	4.2e-122	86%	Mus musculus m31309 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 318760.5
OST2401	gb AA049859	3.7e-83	91%	Mus musculus m31301 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 475859.5. Similar to SH-Y06 SCHMO Q10106 HYPOPHYSICAL 29.7 KD PROTEIN C1405.06 IN CHROMOSOME 11
OST2416	gb U14179	3.1e-54	94%	Mus musculus m31306 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 316711.5. Similar to SH-Y06 SCHMO Q10106 HYPOPHYSICAL 29.7 KD PROTEIN C1405.06 IN CHROMOSOME 11
OST2418	gb U25844	1.1e-47	81%	Mus musculus m31306 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 316711.5. Similar to SH-Y06 SCHMO Q10106 HYPOPHYSICAL 29.7 KD PROTEIN C1405.06 IN CHROMOSOME 11
OST2433	gb AA104747	2.4e-164	91%	Mus musculus m31306 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 316711.5. Similar to SH-Y06 SCHMO Q10106 HYPOPHYSICAL 29.7 KD PROTEIN C1405.06 IN CHROMOSOME 11
OST2442	gb U055819	1.7e-56	91%	Mus musculus m31306 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 316711.5. Similar to SH-Y06 SCHMO Q10106 HYPOPHYSICAL 29.7 KD PROTEIN C1405.06 IN CHROMOSOME 11
OST2443	gb AA061741	1.0e-38	94%	Mus musculus m31306 r1 Soares mouse embryo NMHE13.5 14.5 Mus musculus CDNA clone 316711.5. Similar to SH-Y06 SCHMO Q10106 HYPOPHYSICAL 29.7 KD PROTEIN C1405.06 IN CHROMOSOME 11
OST2455	gb AA167801	4.2e-62	90%	Homo sapiens sq38c06 r1 Scratogene INT neuron (4937233) Homo sapiens CDNA clone 611978.5
OST2459	gb U051333	1.4e-119	96%	Mus musculus Mus musculus co-chaperonin 'culcator' A, mRNA, complete cds
OST2464	gb U052623	1.8e-116	94%	Mus musculus m345h09 r1 Soares mouse complete cds

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Figure 8 cont'd.

OST2829	gb AA002649	7.7e-90	941	LPS-binding protein Mus musculus m81906.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 426106 5' similar to SR:MSK5_HUMAN 000587 SERUM PROTEIN MS155. [13]
OST2814	gb U07692	1.4e-222	971	mus musculus Mus musculus (H-terminal) asparagine amidohydrolase (Hcaul) mRNA, complete cds
OST2815	gb AA060795	2.1e-89	971	Mus musculus m75005.r1 Soares mouse p3HM19.5 Mus musculus cDNA clone 482313 5' similar to WP:42110.4
OST2819	gb AA163971	6.0e-61	701	CE00166 CNP Mus musculus m460.01.r1 Life Tech. mouse embryo 13 5dpc 1066014 Mus musculus cDNA clone 554427 5'
OST2822	gb U04515	6.1e-64	911	Mus musculus m49140.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 361868 5' similar to p3HM19.5
OST2827	gb J03583	1.3e-66	931	ADP-RIBOSYLATION FACTOR-LIKE PROTEIN 3 Rattus norvegicus rat clathrin heavy chain mRNA, complete cds
OST2803	gb U04850	4.8e-75	931	Mus musculus m42002.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 353067 5' similar to gb U12248
OST2821	gb U07798	1.4e-125	981	Mus musculus C57BL/6J ribosomal protein 528 mRNA, complete (ribosome) Mus musculus m410.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 425218 5' similar to
OST2827	gb U11047	7.9e-112	971	POLYMERASE V131-LIVER/HEAT Mus musculus m47810.r1 Soares mouse embryo NMH13.5 Mus musculus cDNA clone 316819 5'
OST2909	gb AA162258	8.9e-120	941	Mus musculus m43909.r1 Life Tech mouse embryo 13 5dpc 1066014 Mus musculus cDNA clone 554427 5'
OST2911	gb U073478	1.4e-117	811	Mus musculus Mus musculus acicula nuclear phosphoprotein p32 mRNA, complete cds
OST2914	gb U12236	4.0e-116	951	Mus musculus Mus musculus ANK alpha M290 integrin mRNA, complete cds
OST2916	gb D77002	1.4e-67	921	Mus musculus Mouse embryonal carcinoma F9 cell cDNA, 91810
OST2921	gb W57540	8.4e-106	981	Mus musculus m55506.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 391379 5'
OST2922	gb D50544	8.4e-135	881	Homo sapiens human lymphocyte mRNA for TFIID subunit p22, complete cds
OST2923	gb W85631	3.2e-108	971	Mus musculus m27100.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 407209 5'
OST2926	gb W59561	6.3e-144	941	Mus musculus m47200.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 373968 5'
OST2929	gb W5735	3.0e-92	921	Mus musculus m50112.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 350983 5'
OST2934	gb W82904	1.8e-75	931	Mus musculus m44611.r1 Beckington mouse embryonic region Mus musculus cDNA clone 540788 5' similar to
OST2940	gb AA154035	1.4e-114	971	gb:X83590 M. musculus mRNA for ribosomal protein L5, 3'-end
OST2942	gb U04802	1.4e-91	961	Mus musculus m40405.r1 Soares mouse p3HM19.5 Mus musculus cDNA clone 350960 5'
OST2948	gb AA104292	5.1e-32	811	Rattus norvegicus EST0035 rat lambda ZAP11 library (C.P. Hamel) Rattus norvegicus cDNA clone pCOV 5' similar to ADP-ribosylation factor (ARF)-like protein
OST2951	gb W10606	1.8e-97	981	Mus musculus m44611.r1 Soares mouse p3HM19.5 Mus musculus cDNA clone 313557 5' similar to PIN:J5073H J5073H Affrase inhibitor protein precursor, mitochondrial
OST2954	gb AA013172	3.1e-137	971	Mus musculus m76007.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 421017 5'
OST2963	gb U04744	4.2e-11	801	embryo NMH13.5 14.5 Mus musculus cDNA clone 479149 5' similar to WP:45812.4 CE02740
OST2971	gb AA120487	9.2e-107	101	Homo sapiens h27908.r1 Soares fetal lung NMH1919 Homo sapiens cDNA clone 298766 5'
OST2974	gb U03353	2.6e-102	881	Mus musculus m412107.r1 Beckington mouse embryonic region Mus musculus cDNA clone 537733 5' similar to SK-Y005-YEAST P30219 HYPOTHETICAL 44.2 AD PROTEIN IN SC02-HMF1 INTERGENIC REGION
OST2977	gb X07325	6.1e-164	971	Rattus norvegicus Rattus norvegicus neuroglycan C precursor mRNA, complete cds
OST2981	gb AA206420	1.2e-71	851	Mus musculus M. musculus mRNA HST Homo sapiens h25102723 Homo sapiens lung epithelial cell cDNA clone 440993 3' similar to TR:G972006 G972006 mRNA, EXPRESSED SEQUENCE TAG
OST2983	gb U049206	1.1e-119	901	Mus musculus m49140.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 355842 5' similar to PIN:544900 544900 ZK652.10 protein - Cenohedictia Segans
OST2987	gb AA027603	2.3e-114	961	Mus musculus m412107.r1 Soares mouse embryo NMH13.5 Mus musculus cDNA clone 463771 5'
OST2988	gb X52129	2.2e-52	731	Mus musculus domesticus Mouse testis-specific mRNA rB56.2
OST2989	gb AA152050	1.3e-46	781	Homo sapiens h14812.r1 Soares pregnant uterus NMH1919 Homo sapiens cDNA clone 505151 5' similar to gb:M00156 cdc21 TRANSCRIPTION FACTOR BTF3 (HUMAN)
OST2991	gb AA003171	8.4e-151	931	Mus musculus m45609.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 437057 5' similar to gb:M24194 GUANINE NUCLEOTIDE-BINDING PROTEIN GUA-300UNIT-LIKE PROTEIN (HUMAN); gb:X72313 M. musculus Homo sapiens h072112.r1 Homo sapiens cDNA clone 38905 5' similar to SP:VILL1.CHICK P02640
OST2994	gb H51546	1.9e-51	831	Mus musculus M. musculus mRNA for S100 calcium-binding protein A13 Mus musculus House 3'-directed cDNA, MUSC01209, clone mC0115 Mus musculus Mus musculus lens major intrinsic protein (MIP) mRNA, complete cds
OST2996	gb X99931	1.6e-82	101	Mus musculus m42102.r1 Life Tech mouse embryo 13 5dpc 1066014 Mus musculus cDNA clone 554427 5' similar to gb:215030_rnal MYOSIN REGULATORY LIGHT CHAIN 2, VENTRICULAR (HUMAN); gb:X65979 M. musculus PLKLC-A mRNA for pyosin light chain 2 (HOUSE)
OST2998	gb D19012	3.2e-48	101	Mus musculus m5310.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 467226 5' similar to PIN:28237 528237 NADH dehydrogenase
OST3003	gb U27502	1.3e-169	971	Mus musculus m31402.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 476762 5' similar to SH:AP17_RAT 00180 CLATHRIN COAT ASSEMBLY PROTEIN AP17
OST3004	gb AA103385	1.9e-162	981	Mus musculus Mouse DNA for small GTP-binding protein 510, exon2 and complete cds
OST3010	gb U03277	2.2e-235	991	Mus musculus Mus musculus CTF synthetase homolog (CTPell) mRNA, complete cds
OST3012	gb U09185	2.1e-76	991	Mus musculus Mus musculus large ribosomal subunit protein mRNA, complete cds
OST3015	gb U08651	1.8e-115	901	Mus musculus m4105.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 421017 5'
OST3017	gb AA050908	4.8e-123	921	Mus musculus m4105.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 476762 5' similar to SH:AP17_RAT 00180 CLATHRIN COAT ASSEMBLY PROTEIN AP17
OST3018	gb U03277	2.2e-235	991	Mus musculus Mouse DNA for small GTP-binding protein 510, exon2 and complete cds
OST3019	gb U09185	2.1e-76	991	Mus musculus Mus musculus CTF synthetase homolog (CTPell) mRNA, complete cds
OST3019	gb U08651	1.8e-115	901	Mus musculus Mus musculus large ribosomal subunit protein mRNA, complete cds
OST3017	gb W09096	4.5e-14	741	Mus musculus m4105.r1 Soares mouse embryo NMH13.5 14.5 Mus musculus cDNA clone 421017 5'

Figure 8 cont'd.

UST3105	gb U041453	1.0e-106	871	Mus musculus House mAb
UST3112	gb U04109	9.7e-59	661	Mus musculus Mus musculus
UST3121	gb U041643	1.2e-132	911	prepro-neurexin mRNA, complete cds
UST3124	gb U041199	2.2e-51	871	Mus musculus House mAb
UST3125	gb U028876	6.5e-103	941	protein (nuclein) protein with RNA-binding ability, complete cds
UST3145	gb U0418210	2.2e-52	941	Mus musculus House mAb
UST3152	gb U0409549	4.9e-63	771	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3154	gb U040630	9.1e-69	921	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3155	gb U0409105	4.1e-40	821	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3166	gb U04122835	2.1e-85	691	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3170	gb U0407058	4.6e-106	941	Mus musculus mAb1009, r1 Soares mouse
UST3171	gb U0411107	1.5e-50	711	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3172	gb U0404859	2.2e-134	991	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3175	gb U0403237	4.0e-44	101	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3176	gb U0403147	4.2e-103	991	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3188	gb U050264	1.9e-117	981	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3190	gb U0404022	1.6e-46	781	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3193	gb U0403200	1.7e-208	911	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3194	gb U04168895	6.3e-109	981	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3197	gb U0403187	3.3e-35	911	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3198	gb U0411116	1.1e-105	801	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3199	gb U04189339	3.4e-37	881	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3201	gb U051858	7.9e-66	771	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3202	gb U050426	7.1e-53	961	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3203	gb U0407064	9.0e-166	971	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3204	gb U0418211	4.2e-114	971	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3205	gb U0418567	9.4e-100	871	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3206	gb U040906	1.0e-121	951	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3107	gb U037446	1.1e-114	921	Mus musculus M. musculus Olf1 mRNA for outer dense fiber protein of sperm tails
UST3148	gb U0403024	1.4e-75	861	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3149	gb U0405518	4.7e-139	921	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3149	gb U041666	1.1e-138	971	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3150	gb U0423403	2.1e-180	981	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3151	gb U0459853	6.9e-54	901	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3152	gb U040883	1.9e-173	991	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3158	gb U0423458	2.0e-119	901	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3156	gb U0414441	5.4e-177	901	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3157	gb U04015044	5.5e-114	971	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3158	gb U04061165	6.3e-99	911	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3159	gb U0437356	3.7e-70	871	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3160	gb U0418893	6.7e-34	801	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3161	gb U0437150	5.7e-31	831	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3162	gb U0431148	4.0e-103	841	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3163	gb U0408748	1.9e-129	971	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3164	gb U0403386	7.9e-132	971	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3165	gb U0437385	5.1e-64	991	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3166	gb U0400004	2.8e-48	781	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3167	gb U045236	2.4e-113	911	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3168	gb U04080212	6.0e-90	931	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3169	gb U0474622	1.1e-39	761	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3170	gb U043120	1.5e-14	971	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3171	gb U040074	3.6e-138	891	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3172	gb U0415062	2.3e-107	901	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3173	gb U042756	4.9e-119	841	mouse protein, human mAb for KIAA0045 gene, complete cds
UST3174	gb U0414994	5.4e-101	851	mouse protein, human mAb for KIAA0045 gene, complete cds

[illegible]

Figure 8 cont'd.

OST4196	gb AA11301	3.1e-39	99%	Mus musculus mcl3h06.r1 Soares mouse p10MP19.5 Mus musculus cDNA clone 351323 5'.
OST4223	gb AA203787	2.7e-89	90%	Mus musculus mcl3h06.r1 Soares mouse lymph node N14H1 Mus musculus cDNA clone 643823 5'.
OST4228	gb SS1016	9.3e-205	92%	Bos taurus E2125Kmultiquilinating enzyme [cathepsin, thymus, mRNA, 825 nt]
OST4229	gb Z1263	4.8e-70	97%	Mus musculus M. musculus expressed sequence tag M12577
OST4235	gb W3187	3.0e-173	97%	Mus musculus mcl3h06.r1 Soares mouse embryo NME13.5 14.5 Mus musculus cDNA clone 368820 5' similar to MP:C3205.9 C01849
OST4243	gb AA048921	2.3e-40	86%	Mus musculus mcl3h06.r1 Soares mouse embryo NME13.5 14.5 Mus musculus cDNA clone 479276 5' similar to gb:U13705
OST4245	gb H10216	9.9e-80	75%	Mus musculus domestica C57BL/6J plasma glutathione (HOUSE)
OST4247	gb AA023146	1.5e-115	96%	Homo sapiens mcl3h06.r1 Soares mouse placenta (NME13.5 14.5 Mus musculus cDNA clone 455981 5' similar to SM:AAF-HUMAN 004941 INTERSTITIAL MEMBRANE A4 PROTEIN. [1])
OST4251	gb AA070774	8.7e-154	98%	Homo sapiens mcl3h06.r1 Soares mouse fibroblast (H37212) Homo sapiens cDNA clone 529412 3'.
OST4254	gb W4737	2.4e-82	10%	Mus musculus mcl3h06.r1 Soares mouse embryo NME13.5 14.5 Mus musculus cDNA clone 367930 5'.
OST4258	gb AA013789	4.3e-169	90%	Mus musculus mcl3h06.r1 Soares mouse placenta (NME13.5 14.5 Mus musculus cDNA clone 442373 5' similar to PIR:JC2472 JC2472 RE protein - human
OST4281	gb U16175	4.0e-40	63%	Mus musculus Mus musculus chromospondin 3 (Chr3) gene, complete cds and nuclein 1 (Nuc1) gene, complete cds
OST4283	gb AA007519	8.9e-52	81%	Homo sapiens mcl3h06.r1 Soares fetal liver spleen LPLS 51 Homo sapiens cDNA clone 429358 5'.
OST4288	gb AA000024	1.4e-135	96%	Mus musculus mcl3h06.r1 Soares mouse embryo NME13.5 14.5 Mus musculus cDNA clone 425602 5' similar to gb:Z03920_rna2 M. musculus GSHPx gene (HOUSE)
OST4315	gb M18210	6.4e-62	96%	Mus musculus Mouse transcription factor S-11, clone 8511-3
OST4319	gb J04696	2.0e-127	95%	Mus musculus Mouse glutathione S-transferase class mu (GST-5) mRNA, complete cds

OST4371	gb W45926	9.6e-55	94%	Mus musculus mcl3h06.r1 Soares mouse embryo NME13.5 14.5 Mus musculus cDNA clone 354750 5'.
OST4388	gb M13524	2.6e-111	90%	Mus musculus Mouse serum amyloid A pseudogene (p1-304)
OST4393	gb M16778	4.7e-45	82%	Homo sapiens mcl3h06.r1 Soares mouse cDNA clone 128530 3'.
OST4002	gb AA000314	1.9e-112	96%	Mus musculus mcl3h06.r1 Soares mouse embryo NME13.5 14.5 Mus musculus cDNA clone 425700 5'.
OST4003	gb L37297	2.9e-121	91%	Mus musculus Mus musculus (clone 86) myeloid secondary granule protein mRNA
OST4011	gb L26664	2.0e-155	94%	Mus musculus EST P013
OST4028	gb 0087470	7.5e-93	92%	sequence tag EST P013
OST4033	gb AA084704	2.2e-54	88%	Homo sapiens mcl3h06.r1 Soares mouse gene, partial cds
OST4051	gb P03500	7.6e-63	86%	Homo sapiens mcl3h06.r1 Soares mouse neuron (H37212) Homo sapiens cDNA clone 346559 3' similar to TR:G800529
OST4061	gb W00618	3.1e-118	97%	CA00523 NADH UNICOUPLONE OXIDOREDUCTASE SUBUNIT
OST4070	gb W3515	6.0e-135	94%	Homo sapiens mcl3h06.r1 Soares mouse sequencer clone 14-11008
OST4073	gb K82021	2.0e-105	91%	Mus musculus mcl3h06.r1 Soares mouse p10MP19.5 Mus musculus cDNA clone 315398 5'.
OST4074	gb 063704	3.3e-140	86%	Mus musculus mcl3h06.r1 Soares mouse Rattus norvegicus R. norvegicus mRNA for heat shock related protein
OST4106	gb W75804	1.1e-84	93%	Rattus norvegicus Rat mRNA for dihydropyridinase, complete cds
OST4114	gb W20730	6.5e-90	96%	Mus musculus mcl3h06.r1 Soares mouse embryo NME13.5 14.5 Mus musculus cDNA clone 400594 5'.
OST4121	gb AA044274	2.4e-33	69%	Mus musculus mcl3h06.r1 Soares mouse p10MP19.5 Mus musculus cDNA clone 337296 5'.
OST4134	gb H11489	3.0e-84	85%	Homo sapiens mcl3h06.r1 Soares mouse pregnant uterus NBPV Homo sapiens cDNA clone 484677 3'.
OST4140	gb W71052	3.7e-121	91%	Rattus sp. EST10556 Rattus sp. cDNA 3' end
OST4142	gb C07091	5.7e-74	89%	Mus musculus mcl3h06.r1 Soares mouse embryo NME13.5 14.5 Mus musculus cDNA clone 388729 5' similar to
OST4144	gb X56135	4.4e-41	83%	SM:YB18 YEAST P3182 HYPOTHETICAL 13.6 MD PROTEIN IN PET112-IL51 INTERGENIC REGION. [1])
OST4148	gb W454510	1.5e-134	91%	Rattus norvegicus similar to none
OST4149	gb U26393	2.6e-111	96%	Mus musculus Mouse mRNA for prothymosin alpha
OST4154	gb X56046	1.3e-161	96%	Mus musculus mcl3h06.r1 Soares mouse embryo NME13.5 14.5 Mus musculus cDNA clone 367841 5' similar to PIR:A56059
OST4155	gb X05900	3.5e-58	85%	A56059 protein-tyrosine-phosphatase
OST4166	gb U53859	8.0e-169	90%	Mus musculus Mus musculus transcription factor TFEB mRNA, partial cds
OST4174	gb U11395	1.3e-38	84%	Mus musculus Mouse mRNA (clone lambda-16) for hypothetical protein A
OST4191	gb X63507	2.0e-75	81%	Rattus norvegicus Rat mRNA for lens
OST4192	gb W65157	2.2e-83	82%	betab1-crystallin (p1beta 81-3)
OST4194	gb W4635	8.9e-38	87%	Rattus norvegicus Rattus norvegicus calpain small subunit (csm1) mRNA, partial cds
				Mus musculus Mus musculus X inactive specific transcript (Xist) gene, cosmid MB4-10A, fragment 2
				Mus musculus mcl3h06.r1 Soares mouse embryo NME13.5 14.5 Mus musculus cDNA clone 408455 5' similar to
				SM:GLYM HUMAN P34897 SERINE HYDROXYMETHYLTRANSFERASE, HETEROLOGICAL
				Mus musculus mcl3h06.r1 Soares mouse p10MP19.5 Mus musculus cDNA clone 350148 5'.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17791

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/68; C12N 5/02, 5/06, 15/00, 15/64; C07H 21/04

US CL : 435/6, 320.1, 325, 357; 536/23.1, 24.2; 800/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 320.1, 325, 357; 536/23.1, 24.2; 800/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SAUER, B. Site-specific recombination; developments and applications. Current Opinion in Biotechnology. May 1994, Vol. 5, pages 521-527, see the entire article.	1-8, 10, 20 and 28
Y	SEKINE et al. Frameshifting is required for production of the transposase encoded by insertion sequence 1. Proc. Natl. Acad. Sci. USA. June 1989, Vol. 86, pages 4609-4613, see especially "Frameshifting in Other Systems", page 4613.	10
X	WANG, et al. High frequency recombination between loxP sites in human chromosomes mediated by an adenovirus vector expressing Cre recombinase. Somatic Cell and Molecular Genetics. 09 March 1996, Vol. 21, No. 6, pages 429-441, see especially the abstract.	8

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z

document member of the same patent family

Date of the actual completion of the international search

30 JANUARY 1998

Date of mailing of the international search report

02 MAR 1998

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17791

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ODELL et al. Site-directed recombination in the genome of transgenic tobacco. Molecular and General Genetics. 11 October 1990, Vol. 223, pages 369-378, see especially Figure 1 and the "Result" section.	1-8, 10, 20
X	DYMECKI, S. A modular set of Flp, FRT and LacZ fusion vectors for manipulating genes by site-specific recombination. Gene. 01 June 1996, Vol. 171, pages 197-201, see especially Figure 1.	10
X	HAAS et al. TnMax - a versatile mini-transposon for the analysis of cloned genes and shuttle mutagenesis. Gene. 11 August 1993, Vol. 130, pages 23-31, see especially the abstract.	8
Y	WO 88/01646 (ALLELIX INC.) 10 March 1988 (10.10.88), see especially pages 1-3.	1-8, 10 and 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17791

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-8, 10, 20 and 28

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s) 1-7, 8, 10, 20 and 28, drawn to a library of cultured eucaryotic cells made by a process comprising treating a group of cells with a vector that mediates the splicing of a foreign exon internal to a cellular transcript, the use of the cell from the library to generate a non-human transgenic animal, and the method of making the cell comprising the vector and the use of the vector to make the library of cultured eukaryotic cells.

Group II, claim(s) 9, 11-18, drawn to a vector construct for replacing the 3' end of an animal cell transcript with a foreign exon.

Group III, claim(s) 19, 21 and 22, drawn to the use of a vector according to claim 9.

Group IV, claim 23, drawn to a stably transduced animal cell that incorporates the vector of claim 16.

Group V, claims 24-27, drawn to a method of altering a region of DNA by adding or deleting DNA.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the first group contains the product, a library of cultured eukaryotic cell, a method of using the cells to produce a non-human transgenic animal and a method of making the cells. The additional groups are directed to different vectors having different compositions than the vector used in the first group, cell lines containing those vector constructs and methods of altering the cellular genome. The first group contains a vector having a different composition than the other vectors and therefore the special technical feature present in the first group does not occur in the other groups.

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